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Effects of Starch Addition on Low Fat Rennet Curd Properties and Their Partitioning Between Curd and Whey

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EFFECTS OF STARCH ADDITION ON LOW FAT RENNET CURD PROPERTIES

AND THEIR PARTITIONING BETWEEN CURD AND WHEY

by

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ABSTRACT

Effects of Starch Addition on Low Fat Rennet Curd Properties

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by

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This study determined the impact of starches on the properties of low fat rennet curd as measured by microstructural and instrumental analysis. In experiment 1, 17 starches were initially screened for swelling power, impact of curd yield at 5 g/L and 10 g/L in milk, and settling in rennet-induced partially acidified low fat curd.

Starches examined were narrowed down to five in experiment 2; they included: modified waxy corn starch, waxy rice starch, instant tapioca starch, dextrin, and a modified tapioca starch. These starches were added to skim milk to make rennet-induced partially acidified milk gels. Gels were made by adding starch to skim milk, heating to gelatinize starch, followed by addition of rennet and glucono-delta-lactone (GDL) to induce gelation. Once gels were set they were cut and centrifuged to sediment the curd. The amount of starch lost in whey was quantified to estimate starch retention in the curd. Confocal laser scanning microscopy was used to determine starch impact on curd microstructure. Curds yields were 13.1%, 18.4%, 20.7%, 21.5%, 23.5%, and 13.2% for

control gel, and gels containing waxy corn, waxy rice, instant tapioca, modified tapioca and dextrin starches, respectively. Estimated starch retentions in the curds were 71%, 90%, 90%, 21%, and 1% for these curds. Waxy corn, waxy rice, and instant tapioca starches have the potential to improve the texture of low fat cheese because they are retained well in the protein network during coagulation and concentration of the milk proteins, and they generate interruptions in curd network that may help limit extensive protein-protein interactions. Modified tapioca starch causes the protein structure of the curd to be very loose, but it was not retained optimally in the curd. Also, because there were few distinct starch particles in the modified tapioca curd network, it is likely that when it is subjected to all the cheesemaking steps the same loose protein structure would not be observed. Dextrin was not retained well in the curd, nor did it disrupt the protein network, making it unsuitable for use in low fat cheese.

In experiment 3, low fat cheddar cheeses were made with waxy corn starch, waxy rice starch, modified tapioca starch, and instant tapioca starch. Modified tapioca starch did not increase the moisture content of the cheese. Waxy corn starch, waxy rice starch, and instant tapioca starch all increased the moisture content of the cheeses significantly. However, when moisture contents of cheeses were over 61%, the body of the cheese visibly softened during storage, making the cheese very pasty. When starch-containing cheeses had moisture levels lower than that, the curd did not knit together well.

(95 pages)

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CHAPTER 1

GENERAL INTRODUCTION

With increasing levels of consumer health awareness and the rising rates of obesity in the United States, there is more interest in the development of healthier food products. Formulating reduced calorie or reduced fat foods with the quality and acceptability of their full-calorie or full fat counterparts has long been an issue for food processors. Cheese is one such food where formulating an acceptable low fat product has been a challenge (Johnson et al., 2009). Texture of cheese is one of the most important factors determining cheese quality and in many evaluations low fat cheese texture has been rated as unacceptable. Development of low fat natural cheese with a texture similar to that of full fat cheese is essential for consumer acceptance. A recent consumer preference study indicated that consumers are unwilling to sacrifice texture of cheese even if the product is lower in fat and calories (Drake et al., 2009).

Addition of starch to low fat cheese is a promising approach to improving the texture of low fat cheese. The texture of low fat cheese is largely determined by the homogenous protein network that provides few fracture points for adequate chew down properties. Starch inclusion into cheese curd may generate discontinuities in the matrix, weakening the protein structure, thus improving cheese texture. Because of the large selection of starches available, determining which has the best properties to improve low fat cheese texture is imperative to formulating an acceptable product. The ideal starch would increase the openness and moisture content of low fat cheese, and be retained in the curd rather than lost in the whey. By selecting starches that are retained well in the curd, the amount of starch that must be added to the cheese milk to achieve the desired effect is minimized. Also, whey is a valuable by-product of cheese making, and if too much starch is lost in the whey the integrity of the whey is compromised. This research serves as a preliminary step in understanding how starches behave in low fat cheese by determining the influence of several starches on rennet-curd yields and measuring their partitioning between curd and whey.

CHAPTER 2

LITERATURE REVIEW

According to the U.S. Food and Drug Administration (Anonymous, 2009), to label a food as low fat it must contain no more than 3 g fat per reference amount customarily consumed, and per 50 g if references amount is ≤30 g, as it is for cheese. For cheese to be labeled low fat it must contain 6% or less fat. Several reviews have been published summarizing the research that has been done with low fat cheese (Drake and Swanson, 1995; Rodriguez, 1998; Mistry, 2001; Banks, 2005; Johnson et al., 2009).

TEXTURE OF LOW FAT CHEESE

Texture is identified by consumers as one of the most important factors determining low fat cheese quality (Gwartney et al., 2002), and in many evaluations low fat cheese texture has been rated as unacceptable (Banks et al., 1989; Banks, 2005). Rubberiness, increased hardness, dryness, and crumbliness are common defects associated with fat-reduction (Adhikari et al., 2003; Banks, 2005). Descriptors for low fat cheese texture include: dry, crumbly, overfirm, rubbery, hard, dry, grainy, waxy, fracturable, chewy, hard, springy, less sticky, less meltable, and less cohesive (Banks et al., 1989; Gwartney et al., 2002; Banks, 2005).

The role of fat in full fat cheese texture is not completely understood. It is postulated that in addition to giving a creamier, mouth-coating feel to cheese, it also imparts discontinuity to the protein matrix which is functionally important. These discontinuities create weak spots in the protein matrix that improve the chew-down properties of cheese (Johnson et al., 2009). Texture attributes are influenced primarily by the change in protein matrix resulting from fat removal (Bryant et al., 1995). Texture of cheese is the result of intricate interactions among its components including fat, moisture, protein, calcium, and pH. Reduction of fat in addition to necessary changes in manufacture of low fat cheese alters ratios of these components, resulting in the decrease in texture acceptability.

Measures of texture as determined by two-bite texture profile analysis (TPA) include hardness, gumminess, springiness, cohesiveness, adhesiveness and chewiness. All of these parameters are shown to increase significantly as fat content of cheese is lowered (Bryant et al., 1995; Awad et al., 2005). Even when moisture in the non-fat substance (MNFS) is kept the same between lower-fat and full fat cheese, the lower-fat cheeses are considerably firmer and more elastic, likely because reduced-fat cheeses have 30% more protein matrix than their full fat counterparts, which must be cut or deformed in sensory and texture assessments (Emmons et al., 1980; Mistry and Anderson, 1993). In reduced-fat cheddar aged 3 to 6 months, hardness and fracturability increased concomitantly with moisture loss as a result of casein degradation, which increases protein-protein interactions, and also the amount of free amino acids and small peptides that bind free water and strengthen the casein matrix (Dabour et al., 2006).

Microscopic observations also provide valuable information on cheese texture. Microstructure analysis by Bryant et al. (1995) of 5 different, 4 month-old, Cheddar cheeses with 13% to 34% fat demonstrated that lowering fat level caused a loss in the open, intricate structure in full fat cheeses. Dabour et al. (2006) described low fat cheese microstructure as a stretched protein matrix with few fat globules scattered between.

MODIFICATION OF TEXTURE

Several methods can be employed to modify and improve low fat cheese texture. Alteration of cheese-milk processing conditions, modification of make procedure (Banks et al., 1989; Mistry, 2001; Dabour et al., 2006; Johnson et al., 2009), and inclusion of additives (Aryana and Haque, 2001; Ma et al., 1997) have all been studied.

Alteration of Cheese Milk

Use of ultrafiltered milk for low fat cheesemaking has been investigated (De Boer and Nooy, 1980; McGregor and White, 1990; Drake and Swanson, 1995). Drake and Swanson (1995) concluded that while low fat cheddar and mozzarella cheeses made from ultrafiltered milk exhibited an increased moisture content, they failed to show an improved texture compared to control low fat cheeses. The same study also looked at homogenization of milk before cheese-making and found that it did increase moisture content and improve texture as measured by TPA of low fat cheese as compared to low fat control cheese. However, despite the differences being statistically significant, they were not of commercial value, which coincides with the conclusions of Emmons et al. (1980). In other studies, homogenization of just the cream used in low fat cheese was shown to improve the texture, flavor and appearance of the cheese as homogenization decreases the size of the fat globules, but increases the number present making them more evenly distributed through the protein matrix (Metzger and Mistry, 1994, 1995; Madadlou et al., 2007). These differences, unfortunately, are not practically significant as the fat content of cheese is reduced (Johnson et al., 2009). Cheese milk pasteurization temperature also gives improved measurements in instrumental rheology, but has little effect on sensory scoring and acceptability (Guinee et al., 2000).

Preacidification of cheesemilk has been shown to improve the textural characteristics of low fat mozzarella cheese, and reduced fat Kashar cheese (Merrill et al., 1994; Fife et al., 1996; Metzger et al., 2001abc; Kaceli et al., 2006). The calcium content of cheese increases as fat content is decreased, and low fat mozzarella has been shown to have 50% more calcium per gram than normal low moisture part skim mozzarella (Rudan et al., 1999). Acidifying cheese milk prior to cheese-making decreases the final calcium content of low fat cheese, especially the water-insoluble calcium, and as calcium plays an important role in protein crosslinking, reducing its content results in a softer, less chewy product. However, Metzger et al. (2001a) found that, depending on the type of acid used and level of preacidification, the yield efficiency was reduced by 2.2% to 5.5% due to and increased loss of casein and fat in the whey, which limits the potential benefits of the preacidification treatment.

Modification of Make Procedure

Reducing cook temperature and time, shortening stirring time, washing curd, and larger cut size can alleviate some of the defects associated with low fat cheese texture principally by increasing MNFS (Banks et al., 1989; Mistry, 2001; Johnson et al., 2009). Dabour et al. (2006) found that using lower cook temperature and higher pH at salting compared to full fat cheese when making low fat cheddar cheese, allowed the moisture content and the final cheese pH to be increased. Lower cooking temperatures slow whey expulsion from the curd, while higher salting pH reduces time available for whey drainage.

Higher moisture content has been associated with decreased fracturability as determined by TPA. Cheese cohesiveness was shown to decrease as cheese moisture content decreased. Moisture in the non-fat substance should be slightly higher in lower-fat cheese than in full fat cheese to achieve more similar texture (Emmons et al., 1980). Increasing pH of curd milling affected firmness and composition of reduced-fat cheeses but it did not affect grading scores by commercial graders (Guinee et al., 2000). Higher pHs at draining lead to higher calcium content and lower retention of chymosin in low fat cheeses, which contributes to an overfirm texture (Mistry, 2001). Calcium content in a 33% reduced-fat Cheddar is about 30% higher than its full fat equivalent (Nauth and Ruffie, 1995). Although improvements to low fat cheese texture can be achieved through alteration of the cheese make procedure, the improvements are not drastic enough to generate a satisfactory texture.

Inclusion of Fat Replacers

Another more promising approach to improving low fat cheese texture is through the use of fat replacers. Fat replacers are classified either as mimetics or substitutes. Fat substitutes are fat-based, with properties similar to natural fat but with reduced caloric content, while mimetics are carbohydrate- or protein-based that mimic the properties of natural fat (Ma et al., 1997; Rodriguez, 1998). While research on fat substitutes has been performed and several studies show promising results for texture enhancement (Babayan and Rosenau, 1991; Drake et al., 1994ab; Crites et al., 1997; Rudan et al., 1999), many of the substitutes are not approved for use in cheese so the focus has shifted to examining fat mimetics. Several fat-replacers have been developed for specific use in low fat cheese including as shown in Table 1.

Table 1. Descriptions of fat replacers studied for use in low or reduced fat cheeses

Fat Replacer	Description	Supplier
Simplesse	Whey protein based	NutraSweet Co., Deerfield, IL
Dairy-Lo	Whey Protein concentrate with 35% protein	Pfizer Inc., New York, NY
Novagel	Blend of microcrystalline cellulose and guar gum	FCM Corp., Philadelphia, PA
Stellar	Blend of modified corn starch and xanthan gum	A. E. Staley MFG. Co., Decatur, IL

These protein- and carbohydrate-based, dairy and non-dairy fat replacers have been shown to soften low fat cheese by imparting discontinuity to the texture and increasing moisture content (Drake et al., 1996a; McMahon et al., 1996; Ma et al., 1997; Aryana and Haque, 2001). Ma et al. (1997) looked at inclusion in low fat Cheddar cheese of the protein-based fat replacers Simplesse and Dairy-Lo and at Novagel and Stellar, which are both carbohydrate-based fat mimetics. They concluded low fat cheeses with carbohydrate mimetics had structures more similar to full fat cheese than did control low fat cheeses or those made with protein mimetics. Some investigations of the protein-based fat replacers Simplesse and Dairy-Lo suggest that their inclusion in natural, low fat cheeses may improve texture and acceptability as compared to low fat control cheeses (Aryana and Haque, 2001), while others report they have a detrimental or no commercially valuable effect (Ma et al., 1997; Fenelon and Guinee, 1997).

Inclusion of Simplesse and Novagel in low fat white brined cheese improved texture parameters compared to control cheese as determined by TPA, although the differences were not detectable in sensory scoring (Romeih et al., 2002). Another report with low fat white pickled cheese also reported that although fat replacers may improve some texture parameters of low fat cheese, their inclusion alone is not enough to alleviate all texture defects (Kavas et al., 2004). In their study with fresh low fat Kashar Cheese, Koca and Metin (2003) concluded that while Simplesse increased the softness of the cheese, after 90 d consumer acceptability decreased because of excessive softening. In that same study, Dairy-Lo had no effect on texture or sensory properties of the cheese.

Increased softness in low fat cheeses made with fat replacers can be due to the fat replacers creating discontinuities in the protein matrix, or due to the increased moisture level resulting from the high water binding capacity of the fat replacers. Studies with low fat mozzarella show that although addition of Simplesse, Dairy-Lo, Novagel, and Stellar all increase cheese moisture, only the Novagel increased the openness of the protein matrix (McMahon et al., 1996).

Other inclusions to low fat cheeses that have been examined for texture improvement include β-glucan (Konuklar et al., 2004; Vithanage et al., 2008), lecithin (Drake et al., 1996b; Sipahioglu et al., 1999) gum tragacanth (Rahimi et al., 2007), carageenan (Kavas et al., 2004; Totosaus and Guemes-Vera, 2008), pectin (Liu et al., 2008), emulsions (Lobato-Calleros et al., 2008) and exopolysaccharide-producing bacteria (Perry et al., 1997; Broadbent et al., 2001).

STARCH USE IN CHEESE

Types of carbohydrates that could be incorporated into low fat cheese include gums, starches, and dextrins. Starches in particular embody a diverse array of functional attributes that could potentially improve low fat cheese texture. Through specialty modifications such as cross-linking, acid hydrolysis, or substitution, starches can be manipulated to serve almost any function within a food matrix. The use of starch is common in dairy products such as ice cream, yogurt, and imitation cheeses. Starch inclusion in both full and low fat ice cream provides enhanced creaminess, improved mouthfeel, and better freeze-thaw stability (Stanley et al., 1996; Cody et al., 2007). In yogurt it enhances mouthfeel in addition to preventing excessive syneresis. Little research has been published on the inclusion of starch in natural cheeses, however. Studies focus more often on addition of polysaccharides such as gums or protein-based fat replacers rather than starch (Mistry, 2001; Rahimi et al., 2007). The research that has been done on starch inclusion in low fat cheese has provided valuable information on the subject.

Sipahioglu et al. (1999) examined use of a modified tapioca starch alone and in conjunction with lecithin as a texture modifier in reduced-fat feta cheese. Modified tapioca starch increased the moisture content and lowered protein content of reduced-fat feta, in addition to reducing hardness to below that of full fat feta. Scanning electron micrographs of reduced-fat feta with lecithin and modified tapioca starch exhibited a structure similar to that of the full fat feta.

Addition of modified potato starch to low fat white pickled cheese produced desirable texture changes of the cheese during aging, similar to changes that occur during aging of the full fat cheese (Kavas et al., 2004). The product was also judged to be acceptable by sensory panelists. However, in instrumental texture analysis, low fat cheese with modified potato starch was harder, gummier, and chewier than both low and full fat cheese throughout aging. The cheese containing the potato starch had the same dry matter, nitrogen and fat content as the low fat control, implying the moisture content of the starch added cheese was not greater than the low fat control. Starch in the protein matrix can bind water limiting protein hydration thus increasing cheese hardness and possibly chewiness and gumminess. Since no additional moisture was retained in the curd of the starch added cheese, this is more likely to occur. The authors also do not address whether the starch was retained in the curd during cheesemaking, nor do they test for starch content of the finished cheeses.

Bhaskaracharya and Shah (2001) looked at inclusion of two types of maltodextrins and a modified potato starch in low fat mozzarella. They found that the potato starch increased hardness of the cheese and decreased moisture content. The potato starch had a particulate nature and particles were distributed in serum pockets as well as in the protein matrix. During storage the starch particles would swell and remove moisture from the surrounding protein. The maltodextrin-based fat replacers had amorphous gel-like structures that improved texture characteristics of the cheese and increased openness. The authors attributed the differing properties of the cheeses made with the different starches to the starches' sizes, degrees of microparticualtion, and to their interactions with casein.

As aforementioned, Stellar is a blend of modified corn starch and microcrystalline cellulose. Inclusion of this additive to low fat cheddar did not generate discontinuities in the casein matrix, but did soften the cheese, presumably by decreasing the number of protein layers at the fat-protein interface and increasing cheese moisture content from 39% to 51.2% (Aryana and Haque, 2001). Similar results were reported with Stellar addition to low fat mozzarella (McMahon et al., 1996), who also reported Stellar particles were evenly distributed between the protein matrix and serum channels.

Studies have looked at individual types of starches and their effects on cheese texture, but none have screened many types of starches to determine which functions of which starches are desirable to include in low fat cheese. The proposed research would examine many types of starch to determine suitability for use in low fat cheese.

POTENTIAL PROBLEMS

There are several potential problems associated with starch addition to cheese. Because cheese is a dynamic, high moisture system, molecular mobility is not limited. It has been shown that fat globules in full fat cheese migrate and coalesce, and it is well known that caseins undergo hydration, and breakdown processes as cheese ages. Water migration also occurs. Water existing in serum pockets when cheese is first manufactured readily diffuses into the protein network during storage, contributing changes to the body of the cheese (Marcos, 1993). Because water in cheese is not static, starches that are unswollen going into cheese can take up water from the surrounding protein matrix thereby limiting protein hydration contributing to texture defects, as has been seen in dry starch addition to imitation cheeses (Noronha et al., 2007).

Conversely, gelatinized starches in cheese could increase the moisture content of the cheese, but that bound water could also disassociate from the starch during cheese storage causing excessive softening. Retrogradation, a problem usually associated with baked goods or pie fillings, could occur in the starch phase of low fat cheese. Retrogradation is defined as the recrystallization of the starch polymers amylose and amylopectin. Upon cooling of starch solutions, these molecules begin to reassociate, forming junction zones making the starch less soluble. Retrogradation is initiated by the linear amylose molecules recrystallizing first, while recrystallization of amylopectin is a slower process because of the bulkiness of the branched chains but is responsible for greater changes in gel structure and functionality. In starch gels retrogradation is evidenced by a decrease in the gel volume and an increase in free water. Because gelatinized starch is not a system at equilibrium, it is entropically favorable for starch molecules to recrystallize. Water mobility in cheese with added starch will vary depending on the level and kind of starch added, and water mobility plays an important role in cheese functionality.

Studies of bipolymer mixtures made of starch and milk protein indicate that these types of systems are inherently unstable, due to the incompatibility of polysaccharides and proteins (de Bont et al., 2002; Ye, 2008). Phase separation is thus likely to occur. Such phase separation is characterized by areas concentrated in each component. In starch-containing low fat cheese for example, there would be protein-rich areas and starch-rich areas, rather than the starch homogenously distributed within the protein. The desirability of phase separation within a starch-cheese system is unknown. The formation of starch-rich areas may generate desirable discontinuities within the protein matrix. However, concentration of starch also increases the likelihood of starch retrogradation. Phase separation has been documented in imitation cheeses containing native and pre-gelatinized starches (Noronha et al., 2007).

Another issue with adding starch to cheese-milk prior to cheese-making, is quantification of starch retention in the cheese. The method normally used to measure the amount of starch in a food is enzymatic, where starch is first digested with amylase and/or amyloglucosidase, then treated with glucose oxidase, peroxidase and a leuco dye which binds to oxidized glucose. A spectrophotometer is then used to measure the amount of glucose present, facilitating the quantification of starch. The problem with this method is that some starches are resistant to the enzyme hydrolysis including certain types of starch due to the nature of the starch granule, starches that have undergone modifications such as cross-linking or stabilization are also, and retrograded starch (BeMiller, 2003). This method also requires a number of steps for proper sample preparation prior to the enzymatic starch hydrolysis step such as drying and defatting. Other methods have also been used to quantify starches using high pressure liquid chromatography (HPLC), gas chromatography (GC), neat infrared (NIR), and capillary electrophoresis, but these methods are tedious and precise and also require many steps for sample preparation. It has also been suggested that they are more suitable for less-complex food systems (Peris-Totajada, 2004).

Carbohydrates in food are normally not measured, but rather determined by difference. The weight of the other components of the food are measured and subtracted from the foods total weight, and the difference is the weight of the total carbohydrates (BeMiller, 2003). This method could be used to estimate the amount of starch in cheese, as the only other carbohydrate in cheese in any significant amount is lactose, and lactose can be measured enzymatically. However, fat, protein, moisture, ash, and lactose would all need to be measured before an estimate of the amount of starch present could be made, making this method of starch quantification exhaustive. Methods for direct quantification of just carbohydrates have been developed using the phenol-sulfuric acid method, GC, and HPLC, among others (Peris-Totajada, 2004). These methods also have their limitations.

HYPOTHESIS AND OBJECTIVES

Addition of starch into skim milk prior to renneting and acidification can produce a low fat milk gel with discontinuities within the protein matrix. This has the potential for improving the texture of low fat cheddar cheese.

Objective 1

Determine the extent of incorporation of different types of starch into acidified rennet-induced skim milk gels.

Objective 2

Determine starch impact on microstructure of acidified rennet-induced skim milk gels to provide information on interactions between starch and casein.

Objective 3

Investigate the feasibility of using starches to improve the texture of low fat cheese.

CHAPTER 3

CHARACTERISTICS OF RENNET-INDUCED SKIM MILK GELS MADE WITH DIFFERENT STARCHES

INTRODUCTION

Low fat cheese is too hard and elastic to have proper chew down properties. By interspersing the protein network with another polymer particle it may be possible to increase chewability. Starches represent a very diverse group of food additives. Due to the various modifications that can be done to native starches, the functionality of starches can be tailored to suit nearly any application. Starches have the potential to improve the texture of low fat cheese, and the ideal starch would increase the openness of the cheese body, increase the moisture content, and have good retention in the curd.

Several starches were screened to determine which would be best to further examine for suitability in low fat cheese. Rennet-induced, partially acidified skim milk gels were used as the examination medium to model low fat cheese. Starches' water holding capacities, effects on milk gel yield, and tendency to settle out before gelation occurred were all characterized.

MATERIALS AND METHODS

Starches

Seventeen different starches (as shown in Table 2) were obtained from National Starch Food Innovation (Bridgewater, NJ). The starches included 3 cross-linked,

Table 2. Starch descriptions and numerical designation to identify starches in later tables

Numerical Designation	Starch Description
1	Viscosifying waxy corn starch, cross-linked, stabilized, most process tolerant of #1-3
2	Viscosifying waxy corn starch, cross-linked, stabilized, moderate process tolerance compared to #1 and #3
3	Viscosifying waxy corn starch, cross-linked, stabilized, least process tolerant of #1-3
4	Agglomerated instant, viscosifying waxy corn starch, cross-linked, stabilized, more process tolerant than #5
5	Agglomerated instant, viscosifying waxy corn starch, cross-linked, stabilized, less process tolerant then #4
6	Potato starch, very large granules with phosphate side groups
7	Waxy rice starch
8	Corn starch
9	Agglomerated instant tapioca Starch
10	Modified tapioca starch
11	Maltodextrin with specialty modifications
12	Dextrin with specialty modifications
13	Tapioca starch
14	Traditional gelling starch, acid converted
15	Potato starch based, large granules with phosphate side groups
16	Agglomerated instant modified tapioca starch
17	Agglomerated instant waxy rice starch

stabilized, viscosifying waxy corn starches with different process tolerances, 2 agglomerated instant modified corn starches with differing process tolerance, modified potato starch, native potato starch, waxy rice starch, corn starch, tapioca starch, instant tapioca starch, instant rice starch, maltodextrin, dextrin, modified tapioca starch, instant modified tapioca starch, and an acid converted traditional gelling starch.

Starch Swelling

The swelling properties of the 17 starches were measured at 3 different temperatures in duplicate to determine the amount of water each type of starch holds when given different heat treatments based upon Konik-Rose et al. (2001). In 50-ml conical centrifuge tubes, 0.40 ± 0.03 g of starch was added to 40 ml distilled deionized water (~22°C) and heated with agitation to 60, 66 or 72°C. Solutions heated to 60 or

66°C were held for 30 min at their respective temperatures, while those heated to 72°C were cooled immediately. Heat treatments examined were based on the starch manufacturers recommendation for ideal starch gelatinization. Tubes were cooled to room temperature and centrifuged at 2,000 g for 20 min. Following centrifugation, the weights of supernatant and precipitate were weighed and the amount of water held by the starch was calculated.

Skim Milk Gels

Pasteurized (73°C for 15 s) skim milk was obtained from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan, UT) and stored at 4°C until use. Each starch was dispersed in cold milk at 5 g/L and 10 g/L, and heated to induce gelatinization using 3 heat treatments: heated to 63 or 72˚C then immediately cooled, or heating to 63˚C and held for 30 min at 63˚C before cooling. Heat treatments used were selected based on the starch manufacturers recommendations for proper gelatinization conditions.

Two hundred milliliters of each starch-in-milk solution were prepared in duplicate in 250-ml polycarbonate Nalgene centrifuge bottles (Thermo Fischer Scientific, Rochester, NY). Controls containing milk only were also prepared. Milk-starch mixtures were then heated in a 75 or 85˚C waterbath to 63˚C or 72˚C, respectively, over about 20 min, or to 63˚C and held at that temperature for 30 min in a 64˚C waterbath, after which they were cooled within approximately 10 min to 35˚C. Four grams of glucono-δ-lactone (PURAC America, Blair, NB) and 400 µl of diluted chymosin rennet (Maxiren DS, DSM Food Specialties, Parsippany, NJ) diluted with cold distilled deionized water to 65 International milk clotting units/ ml were added to each mixture. Mixtures were then incubated for 30 min at 35˚C to allow coagulum formation and then cut vertically with 3 cuts in each of 2 perpendicular directions. The bottles containing the curd and whey were centrifuged at 250 *g* at 25˚C for 30 min. The whey was decanted immediately after centrifugation and again after 30 min, and its volume measured using a graduated cylinder. Curd yield was calculated on a volume basis and expressed as a percentage. Observations were made and recorded on both curd and whey properties, and 50 ml of whey from each was retained and frozen for further analysis.

Curd Properties

Skim milk gels were evaluated visually for clean separation of curd from whey after centrifugation and appearance of curd texture. Starch settling at the bottom of the bottle was also looked for after incubation, and again after centrifugation. The visual appearance of the whey was also judged for sliminess, viciousness, and visual similarity to control wheys.

Statistical Analysis

Data on curd yield were evaluated at each starch concentration using a two-way factorial design looking at the effects of starch type, heat treatment, and the interaction between starch type and heat treatment. Starch swelling data was also analyzed with a two-way factorial design looking at the same effects. Statistical analysis was performed with SAS 9.0 (SAS Institute, Inc. Cary, NC) using the proc GLM function with Tukey-Kramer adjustment for multiple mean comparisons. Significance was declared at $P < 0.05$, and tendencies up to $P < 0.10$.

RESULTS AND DISCUSSION

Starch Swelling

The effect of heat treatment on the amount of starch swelling was determined for each starch and the results are given in Figure 1. Type of heat treatment was shown to affect the amount of water held by each starch only for starches 15 and 17. Starches were grouped based on their mean swelling values over all heat treatments. Starches with a water-holding capacity of < 1.0 g/g were classified as non-swelling. Low swelling

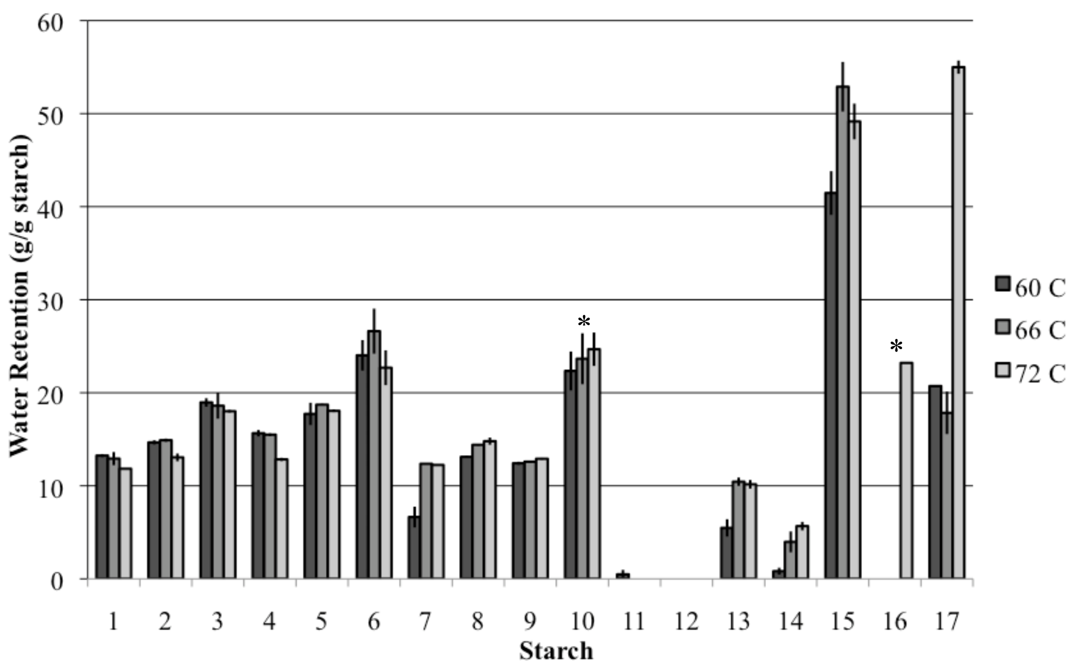

Figure 1. Starch swelling property for starches described in Table 2, measured as grams of water held per gram of starch after centrifugation of starch solutions gelatinized at 60°C for 30 min, 66°C for 30 min, or 72°C. “*” indicates starches separated poorly during centrifugation, therefore swelling values are only estimates or not able to be measured. Error bars show standard error of the mean.

starches held less than 5.0 g/g. Moderate swelling starches held between 5.1 and 15.0 g/g. High swelling starches between 15.1 and 24.0 g/g, and very high swelling starches held greater than 24.0 g/g. Starches 11 and 12 were non-swelling and starch 14 was low swelling. Most starches were moderate swelling and included starches 1, 2, 7, 8, 9, 13, and 17. Starches 3, 4, 5, 6, and 10 were high swelling, and starch 15 was very high swelling.

Heat treatment was only significant for starches 15 and 17 (Appendix B). Starch 15 swelled significantly less with the 60°C heat treatment versus the two higher heat treatments, but with all heat treatments it was still classified as very high swelling. Starch 17 swelled significantly more with the 72°C heat treatment than with the lower heat treatments. With the high heat treatment, starch 17 was classified as very high swelling, while with the other heat treatments it was only high swelling. Starches 10 and 16 separated poorly during centrifugation; therefore the swelling values for those starches are missing or are only estimates.

Curd Yield

Heat treatment had no effect ($P > 0.99$) on curd yield in the control milks without added starch. Control milk curds ranged in curd yield from 11.8 to 14.3%. When starches were added to the milk, the curd yields ranged from 11.0% to 28.5% when 5 g/L starch was added (Figure 2) and 10.8% to 49.8% when 10 g/L starch was added (Figure 3), depending on the type of starch added and the applied heat treatment (Appendix B). Addition of starches 11, 12, and 13 (the dextrin, maltodextrin and tapioca starches) did not significantly increase curd yield at either concentration or heat treatment.

Starch 14 (traditional gelling, acid converted starch) also did not significantly affect curd yield at most concentrations and most heat treatments. Only at 10 g/L and with the 72°C heat treatment did starch 14 significantly increase curd yield over the control.

Conversely, inclusion of starches 1 to 10, and 15 to 17 had significantly higher curd yields than the control, with the effect being more pronounced at the higher starch concentration, and with increasing heat treatment. Higher heat treatment generally increases the starch degree of swelling and as the starch is incorporated into the para-casein network, its associated water is as well, thus increasing curd volume. Inhibition of

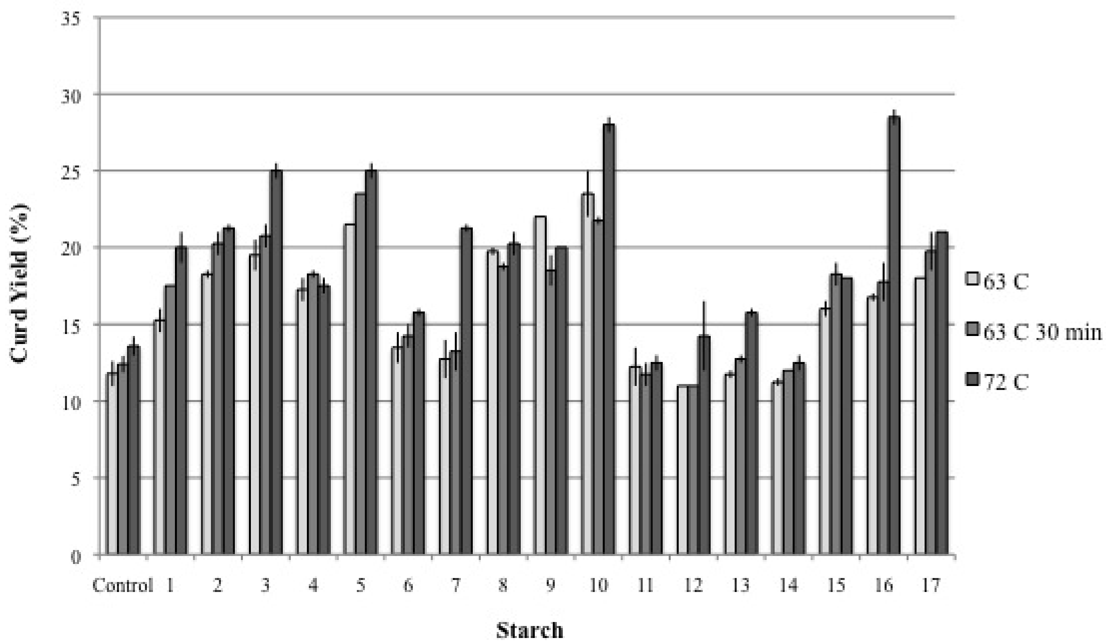

Figure 2. Percent curd yield from rennet acidified milk containing 5 g/L various starches (as described in Table 2) and heated to 63 and 72°C, or 63°C and held for 30 min at that temperature then centrifuged at 250 *g* for 30 min at 25°C. Error bars show standard error of the mean.

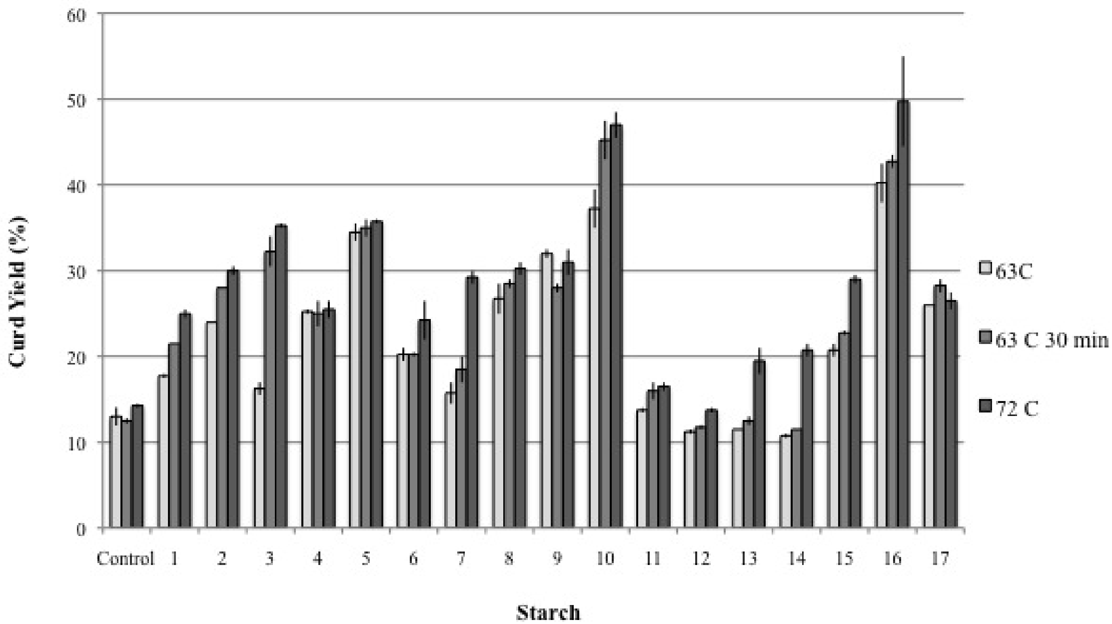

Figure 3. Percent curd yield from renneted acidified milk containing 10 g/L of various starches (as described in Table 2) and heated to 63 and 72°C, or 63°C and held for 30 min at that temperature then centrifuged at 250 *g* for 30 min at 25°C. Error bars show standard error of the mean.

coagulum concentration by the presences of starch also can increase curd volume. Starches 3, 5, 10 and 16 increased curd volume to the greatest extent at 10 g/L with the 72°C heat treatment, bringing the curd yield from 14% with the control to between 35% and 49% when starch was added, a 2.5 to 3.5 fold increase.

Severity of heat treatment did have an effect on curd yields ($P < 0.001$), depending on the type of starch included and its concentration. As previously mentioned, heat treatment did not have a significant effect on control curd yields. It did have a significant effect on curd yields, however, when starches 3, 7, 10, and 16 were added to the milk at 5 g/L, and when starches 3, 7, 10, 13, 14, 15, and 16 were added at 10 g/L. In general, curd yields were highest for these starches at either concentration when milks were given the 72°C heat treatment versus the 63°C or 63°C for 30 min treatments. Only when starch 3 was added at 10 g/L was there a statistically significant difference ($P < 0.05$) in curd yields between the 63°C and the 63°C for 30 min treatment, otherwise there was no difference between those treatments for any of the samples at either starch concentration.

Severity of heat treatment did not have a statistically significant effect ($P > 0.05$) on curd yields when starches 1, 2, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, or 17 were added to milk at 5 g/L, or when starches 1, 2, 4, 5, 6, 8, 9, 11, 12, or 17 were added at 10 g/L. However, despite no measured differences in curd yield when these mixtures were given different heat treatments, heat treatment did influence starch settling out of the milk and in some cases (starches 11, 12, and 13), it was apparent the starch wasn't given adequate heat for proper gelatinization. Cooking starch beyond its temperature of maximum swelling will increase its tendency to settle. Undercooked starch also settles more readily than optimally cooked starch.

Curd Properties

Skim milk gels made with 10 g/L starch had higher curd yields than did those made with 5 g/L using the same starch and temperature parameters. The increase in curd volume could be due to the starch making the curd unable to synerese, but more likely it is just a function of the additional volume from hydrated starch incorporation in the curd. In some cases, too, swollen starch would settle out underneath the curd and its volume would be calculated as curd although it is distinctly separate. Curd properties, however, were better overall with those made at 5 g/L starch rather than 10 g/L; there was less starch settling or separation.

Inclusion of starches 1 to 3, the waxy corn starches, generally produced curd with higher yield than control curds. There was good curd and whey separation with these gels, and there was little to no starch settling during rennet gelation and centrifugation. Starches 4 and 5, the instant waxy corn starches, also produced higher volume gels, but these starches had a tendency to settle out more often than starches 1 to 3 at all heat treatments and concentrations. The wheys of gels made with starches 4 to 5 appeared more viscous and slimy than control whey.

Potato starches 6 and 15 increased curd volumes the most with the higher heat treatments, however, these starches showed lots of setting at both concentrations and with all heat treatments. Corn starch 8 increased the curd volume as compared to control at both inclusion levels and at all heat treatments ($P < 0.001$). Curd with cornstarch was softer than control, and had good separation from the whey, and there was little to no starch settling with all treatment combinations.

Waxy rice starch 7 produced curd with the highest volumes with the 72°C heat treatment. At lower temperatures the starch was not gelatinized and settled out during setting and centrifugation. The higher starch concentration increased the likelihood of starch settling, and kept the cut curd from forming one cohesive mass when heat treated at 72°C. The lower concentration and high heat treatment did produce curd that separated well from the whey, and was more voluminous and softer than the control. Instant rice starch 17 produced curd that showed starch settling at all combinations of heat treatments and concentrations. The wheys of these curds were viscous, and at the 10-g/L inclusion level, there was starch slime on the top of the centrifuged curd.

Starch 13, the regular tapioca starch, increased curd yield only at the highest heat treatment and higher starch concentration but the increase was non-significant ($P = 0.188$). While the starch didn't completely settle out during centrifugation, the curd clearly was softer from starch at the bottom of the bottle, indicating a tendency to demix. At the lower heat treatments, tapioca starch 13 did not gelatinize and settled out during centrifugation. Instant tapioca starch 9 increased curd volume most with the lowest heat treatment, but still increased volume significantly with the other treatments. At both concentrations, curds with this starch were soft and cohesive and there was good curd and whey separation.

Starches 10 and 16, the modified tapioca starches, had the most dramatic visible effect on curd properties. Both prevented the curd from settling during centrifugation and both caused poor curd and whey separation. At 10 g/L, these effects were much more pronounced than at 5 g/L. The whey from these samples was very slimy. Although the starches increased the curd volumes dramatically, the curds were not very cohesive. The higher heat treatments exacerbated these problems.

Maltodextrin and dextrin (starches 11 and 12) inclusion did not significantly change the curd volume at most temperature and concentration combinations. Maltodextrin settled out at all temperature and concentration combinations. Dextrin settled out at both concentrations with the 63°C and 63°C for 30 minute heat treatments. With the 72°C heat treatment, however, no settling was apparent, although, curd volumes were no higher than controls.

The traditional gelling acid-converted starch (starch 14) did not increase curd volume when included at the lower concentration, but at the 10 g/L level and with the highest heat treatment, curd volume was increased. However, curds with this starch were softer at the bottom indicating the starch tendency to settle.

CONCLUSION

For further research, I recommend examining the waxy corn starches 1, 2, 3, corn starch 8, and instant tapioca starch 9 at either concentration and with any of the 3 heat treatments used because these starches produced curds with increased volume, good curd and whey separation, and with minimal starch settling. The waxy rice starch 7 with the 72°C heat treatment and 5 g/L inclusion level also yielded a curd with those properties. Inclusion of dextrin, starch 12, with the 72°C heat treatment also produced curd with good curd and whey separation and minimal starch settling, but it did not increase curd volume. Further examination of this starch would be interesting in that it has a very different effect on curd properties. Also, because modified tapioca starch (10) greatly disrupted the protein interactions in the curd, examining it further with the lower temperature heat treatment and low concentration would yield interesting information on how the starch interacts with the protein.

Due to the experimental constraints of further examining these starches for use in low fat cheese, it is necessary to narrow down the selection to just 5 starches. Because the waxy corn starches (1, 2 and 3) all have very similar properties, I will look at just starch 1, as it will provide adequate information for determining the suitability of starches 2 and 3 in low fat cheese. Looking at starches with different properties will help to determine which properties are most desirable in a starch for use in cheese. Since the dextrin (12), seemed to have little impact on the visual characteristics of the curd as compared to the control, and the modified tapioca starch (10) greatly increased the curd volume and changed the appearance, I will continue to examine these starches. I also will examine waxy rice starch (7) because it has a different botanical source from the other starches selected and is known to have a much smaller granule size, and instant tapioca starch (9) because it has undergone gelatinization and drying steps during its manufacturing that none of the other starches selected have been subjected to.

In further experiments where curd is made according to the model system using milk gels, I will also change the centrifuge speed so that the second whey decant step following centrifugation is not needed. In this chapter the skim milk gels were centrifuged at 250 *g* for 30 min to sediment the curd, while in the next chapter the gels will be centrifuged at 500 *g* for the first 15 min, then the speed increased to 1,000 *g* for the last 15 minutes. This increases the amount of whey expelled initially, and limits the amount expelled once the whey has been decanted, thus eliminating the need for the second decant step.

Also, the heat treatments used in the following experiments will reflect those used in the starch swelling experiments from this chapter rather than those used when making skim milk gels in this chapter. They differed originally as the skim milk gels were made prior to measuring starch swelling, and then the starch manufacturer recommended looking at a wider temperature range when measuring starch swelling.

CHAPTER 4

EFFECTS OF STARCH ADDITION ON LOW FAT RENNET CURD PROPERTIES AND THEIR PARTITIONING BETWEEN CURD AND WHEY

ABSTRACT

Model low fat cheeses were made using 5 different starches at a concentration of 5 g/L in skim milk. Starches used included modified waxy corn starch (WC), waxy rice starch (WR), instant tapioca starch (IT), dextrin (D), and a modified tapioca starch (MT). Milks containing WC, WR, and D were given a 72°C heat treatment, while IT and MT had a 66°C for 30 min treatment. Controls were made for both heat treatments. Milks were renneted, acidified and centrifuged. Curd yields were calculated, and estimated starch content in whey in curd was calculated gravimetrically by alcohol precipitation. Curds yields were 13.1%, 18.4%, 20.7%, 21.5%, 23.5%, and 13.2% for control gel, and gels containing starches WC, WR, IT, MT and D, respectively. Estimated starch retentions in the curds were 71%, 90%, 90%, 21%, and 1% for these curds. Waxy corn, WR, and IT starches have potential to improve texture of low fat cheese because they are retained well in the protein network during coagulation and concentration of the milk proteins, and they generate interruptions in curd network that may help limit extensive protein-protein interactions. Modified tapioca starch causes the protein structure of the curd to be very loose, but it was not retained optimally in the curd. Also, because there were few distinct starch particles in the MT curd network, it is likely that when it is subjected to all the cheesemaking steps the same loose protein structure would not be

observed. Dextrin was not retained well in the curd, nor did it disrupt the protein network, making it unsuitable for use in low fat cheese.

INTRODUCTION

Development of lower-fat foods is often difficult because removal of fat from many food systems results in foods with poor sensorial and functional qualities (Johnson et al., 2009). Low fat natural cheese is one such food where fat reduction gives the cheese a hard, rubbery texture (Drake and Swanson, 1995). Fat has the important role in cheese of imparting discontinuity to the protein matrix, so when fat is removed the cheese is composed of a homogenous, dense protein network (Paulson et al., 1998). Starch inclusion has been suggested as a possible remedy and has even been used in products on the market today such as Cabot 75% reduced fat cheddar (Cabot Cheese, Montpelier, VT)

Starches embody a diverse array of functional attributes that could potentially improve low fat cheese texture. Through specialty modifications such as cross-linking, acid hydrolysis, or substitution, starches can be manipulated to serve almost any function within a food matrix (BeMiller and Whistler, 1996). The use of starch is common in dairy products such as ice cream, yogurt, and processed cheeses (Cody et al., 2007; Trivedi et al., 2008). Starch inclusion in both full- and low fat ice cream provides enhanced creaminess, improved mouthfeel, and better freeze-thaw stability (Stanley et al., 1996; Cody et al., 2007). In yogurt it enhances mouthfeel in addition to preventing excessive syneresis (Sadoval-Castilla et al., 2004).

Studies focus more often on addition of polysaccharides such as gums, or protein-based fat replacers rather than starch (Mistry, 2001; Rahimi et al., 2007). Other inclusions to low fat cheeses that have been examined for texture improvement include β-glucan (Konuklar et al., 2004; Vithanage et al., 2008), lecithin (Drake et al., 1996b; Sipahioglu et al., 1999), gum tragacanth (Rahimi et al., 2007), carageenan (Kavas et al., 2004; Totosaus and Guemes-Vera, 2008), pectin (Liu et al., 2008), emulsions (Lobato-Calleros et al., 2008) and exopolysaccharide-producing bacteria (Perry et al., 1997; Broadbent et al., 2001).

Sipahioglu et al. (1999) examined the use of modified tapioca starch alone and in conjunction with lecithin as a texture modifier in reduced-fat feta cheese. Modified tapioca starch increased the moisture content of reduced-fat feta, in addition to reducing hardness to below that of full fat feta. Scanning electron micrographs of reduced-fat feta with lecithin and modified tapioca starch exhibited a structure similar to that of the full fat feta.

Addition of modified potato starch to low fat white pickled cheese produced desirable texture changes of the cheese during aging, similar to changes that occur during aging of the full fat cheese (Kavas et al., 2004). The product was also judged to be acceptable by sensory panelists. However, in instrumental texture analysis, low fat cheese with modified potato starch was harder, gummier, and chewier than both low and full fat cheese throughout aging. The cheese containing the potato starch had the same dry matter, nitrogen and fat content as the low fat control, implying the moisture content of the starch added cheese was not greater than the low fat control. Starch in the protein matrix can bind water limiting protein hydration thus increasing cheese hardness and possibly chewiness and gumminess. Since no additional moisture was retained in the curd of the starch-added cheese, this is more likely to occur. The authors also do not address whether the starch was retained in the curd during cheesemaking, nor do they test for starch content of the finished cheeses.

Bhaskaracharya and Shah (2001) looked at inclusion of two types of maltodextrins and a modified potato starch in low fat mozzarella. They found that the potato starch increased hardness of the cheese and decreased moisture content. The potato starch had a particulate nature and particles were distributed in serum pockets as well as in the protein matrix. During storage the starch particles would swell and remove moisture from the surrounding protein. The maltodextrin-based fat replacers had amorphous gel-like structures that improved texture characteristics of the cheese and increased openness. The authors attributed the differing properties of the cheeses made with the different starches to the starches' sizes, degrees of microparticualtion, and to their interactions with casein.

As mentioned in Chapter 2, Stellar is a blend of modified corn starch and microcrystalline cellulose. Inclusion of this additive to low fat cheddar did not generate discontinuities in the casein matrix, but did soften the cheese presumably by decreasing the number of protein layers at the fat-protein interface and increasing cheese moisture content from 39% to 51.2% (Aryana and Haque, 2001). Similar results were reported with Stellar addition to low fat mozzarella (McMahon et al., 1996), who also reported Stellar particles were evenly distributed between the protein matrix and serum channels.

The process of making natural cheeses involves the separation of curd and whey, making starch retention in the curd an issue. In order to eventually use starch to improve the texture of low fat natural cheeses, an understanding of starch behavior in a model cheese system is valuable as it shows whether starches interact strongly or weakly with casein matrix. In this experiment, 5 different starches were used to make rennet-induced partially acidified skim milk gels. The partitioning of starch between curd and whey was quantified, and starch impact on microstructure of the milk gels was determined using laser scanning confocal microscopy (LSCM), in which the starch is oxidized to produce aldehyde groups that can then bind a fluorophore (Lehninger, 1975).

MATERIALS AND METHODS

Milk Gel Make Procedure

Five starches were obtained from National Starch (Bridgewater, NJ). They included a modified waxy corn starch (WC), waxy rice starch (WR), instant tapioca starch (IT), dextrin (D), and modified tapioca starch (MT). Moisture content of each starch powder was determined by vacuum oven at 66°C and $1.5x10^3$ torr. Pasteurized (73°C for 15 s) skim milk was obtained from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan, UT) and stored at 4°C until use.

Each starch was dispersed in cold milk at 5 g/L. Two hundred milliliters of each starch-in-milk solution were prepared in 250-ml polypropylene Nalgene centrifuge bottles (Thermo Fischer Scientific, Rochester, NY). Controls containing only milk were also prepared. Milks containing WC, WR, and D were heated to 72°C in an 85°C-waterbath, and those containing MT and IT were heated to 66°C in a 75°C-waterbath and held for an additional 30 min at 66°C to induce starch gelatinization. After the cook-up step, milks were cooled in a cold water bath to 35°C over about 10 min, then 4 g of glucono-δ-lactone (PURAC America, Blair, NE) and 400 µl of diluted double strength chymosin rennet (Maxiren DS, DSM Food Specialties, Parsippany, NJ) diluted with cold distilled deionized water to 65 International milk clotting units/ml were added to each mixture. Mixtures were then incubated in a waterbath for 30 min at 35˚C to allow coagulum formation and then cut vertically with 3 cuts in each of 2 perpendicular directions, then centrifuged (RC5C, Sorvall Instruments, Waltham, MA) for 15 min at 500 *g* at 25°C, then 15 min at 1,000 *g* to sediment curd. The whey was decanted immediately after centrifugation and weighed; curd was also weighed. Curd and whey yields were calculated on a weight basis and expressed as a percentage of the original weight of the milk-starch mixture. A portion of whey was used to measure moisture content (in triplicate), while another portion was frozen and retained for starch analysis. Curd was retained for microstructural analysis.

Starch Loss in Whey

To determine the amount of starch lost in the whey a modified version of the method for total dietary fiber was used (AOAC, 1990). Four whey samples from each starch-containing curd were thawed. Control whey samples from both heat treatments were also used. Approximately 12 g of each whey in duplicate was accurately measured into beakers, and 50 ml of 0.08 M pH 6.0 phosphate buffer was added to each sample, followed by pH adjustment to between 4.0 and 4.6 with 0.275 M HCl. Then 280 ml of 95% ethanol warmed to 60°C was added to each sample and the precipitate allowed to form for 60 min. Coarse fritted glass 50-ml crucibles (Cole Parmer, Vernon Hills, IL) containing 0.5 g of acid washed celite (Sigma Aldrich, St. Louis, MO) were dried over night and weighed before use; prior to filtration the bed of celite was evenly redistributed using 78% ethanol. After 60 min, each sample was filtered through the crucibles, followed by washing with three 20-ml portions of 78% ethanol, two 10-ml portions of

95% ethanol, and two 10-ml portions of acetone. Following filtration, crucibles containing residue were dried overnight in a vacuum oven at 66°C and $1.5x10^3$ torr then weighed.

Performing the analysis with starch in water solutions of known concentration was used to determine recovery of starch after ethanol precipitation. Solutions of each starch were made and given heat treatments identical to the milk used to make the model cheeses. A portion of each solution containing approximately 0.04 g of starch accurately measured on an analytical balance was analyzed as described above. Weights of residues were compared with the known amount of starch in each solution.

Sample Preparation for LSCM Imaging

Curd samples were prepared for microstructural analysis using LSCM by the method of McManus et al. (2009). Curd was cut to 1x1x1 cm cubes and protein was fixed with 1% (wt/vol) osmium tetroxide (Ted Pella Inc., Redding, CA) in whey solution. Samples in fixative were microwaved (Model 3470, Ted Pella Inc., Redding, CA) at high power under vacuum for 4 cycles of 2 min on then 2 min off, while the sample was maintained at 31°C, then stored overnight at 4°C. Prior to imaging, samples were cut into 1-mm thick slices, and placed for 20 min in 0.5% aqueous periodic acid solution (Sigma Aldrich, St. Louis, MO) to oxidize the starch, and washed with several exchanges of deionized distilled water. Next, samples were immersed in 1% aqueous acriflavine HCl (Sigma Aldrich) to stain starch using the same microwave procedure. Samples were washed till little dye was expelled in the wash water, and were next immersed in 0.01% aqueous Rhodamine B (Sigma Aldrich) to stain protein and again microwaved. Samples were again washed, then mounted on a glass slide with glycerol/gelatin (Horobin and Kiernan, 2002) and covered with a coverslip.

Additionally, samples of the starches gelatinized in water with heat treatments identical to those each receives in milk were also prepared to assure each starch could be imaged with the staining procedure used. In 50-ml conical centrifuge tubes, 0.1g of each starch was added to 20 ml distilled deionized water. Starch WC, WR, and D solutions were heated to 72°C, and IT and MT starches were heated to 63°C and held at that temperature for 30 min. Solutions were then cooled to room temperature in a cold water bath, before 0.1 g periodic acid was added to each solution and allowed to oxidize for 20 min. An additional 20 ml of water was added to each solution, then tubes were centrifuged 5 min at 3,000 *g*. Supernatant was decanted and more water added to each tube to bring the volumes back to 40 ml. Tubes were shaken to wash the pelleted material, then centrifuged and decanted again. Five milliliters of 0.3% aqueous acriflavine was added to each tube. Solutions were allowed to stain 10 min, followed by extensive washing following the same protocol for washing out the periodic acid. Once no residual dye was apparent in supernatants, the gelatinized starch pellets were smeared on glass slides. A drop of water was placed on top of each smear, followed by a glass coverslip. Glass coverslips were adhered with a glycerol/gelatin mixture (Horobin and Kiernan, 2002) to seal solutions between the slide and coverslip.

Samples were imaged on a Biorad MRC 23 confocal microscope (Biorad, Hercules, CA) with a Kr/Ar laser exciting the acriflavine at 488 nm and the Rhodamine B at 568 nm. Emissions were from 488 to 650 nm and 550 to 750 nm, respectively, and exclusion filters of 512 to 532 nm and above 585 nm were used to capture the fluorescent signals of acriflavine bound to derivatized starch, and Rhodamine B bound to protein, respectively. Images were false colored with protein as red-orange and starch as yellow-green.

Experimental Design

Moisture contents were measured in triplicate. Curd and whey yields were calculated based on 4 samples per curd type. Alcohol precipitation was done in duplicate for 4 whey samples from each type of curd. Differences in alcohol-insoluble residue for all curd types were determined with SAS 9.0 (SAS Institute, Inc., Cary, NC) using proc GLM with a one-way factorial design, Tukey-Kramer adjustment for mean comparison and significance declared at $\alpha = 0.05$. Recovery factors were determined using 5 samples per starch. Confocal imaging was done on at least 2 subsamples of 3 curd samples for each type of curd. Due to variation in the alignment of the microscope and fastidiousness of the technique, many images of each sample were taken to obtain acceptable images. Formulas for calculations are given in Appendix A.

RESULTS

Alcohol Precipitation

Moisture content of the starch powders determined gravimetrically and their recovery factors after alcohol precipitation are shown in Table 3. There was no significant effect of starch type on recovery and virtually all the starch was recovered and therefore a correction factor was not needed.

Table 3. Dry starch moisture contents and recovery factors from starch alcohol precipitation.

Starch	Moisture Content (%)	Recovery Factor
Waxy Corn Starch	8.92 ± 0.03	1.14 ± 0.01
Waxy Rice Starch	9.59 ± 0.06	1.06 ± 0.07
Instant Tapioca Starch	8.12 ± 0.09	1.07 ± 0.06
Modified Tapioca Starch	10.62 ± 0.32	1.11 ± 0.05
Dextrin	4.10 ± 0.11	0.96 ± 0.05

Starch Fluorescence

Starches WC, WR, and IT when derivatized and stained using the periodic acid-acriflavinemethod fluoresced (Figure 4), making it a suitable method for distinguishing these starches in the skim milk gels. Periodic acid oxidizes some of the glucose residues of the amylose and amylopectin chains at carbons 2 and 3, splitting the bond between the two carbons and converting the hydroxyl groups on each to aldehydes (Lehninger, 1975; McManus et al., 2009). The presence of the dialdehyde then facilitates binding to the starch by the pseudo-Schiff base acriflavine. The strongest fluorescence was observed around the residual starch granules, presumably because of the greater presence of oxidized glycol units that can bind the acriflavine. Amylose leached from granules would also bind acriflavine, but because of extensive rinsing steps during sample preparation soluble amylose molecules would be removed. Images for all 5 starch gels in water are shown in Figure 4. Both the MT and D starches should have no granular structure, so the particles seen in the images are probably unhydrolyzed material present in the starch powders. During preparation of these samples, there was virtually no starch pellet following staining, washing, and centrifugation steps of the D and MT starches.

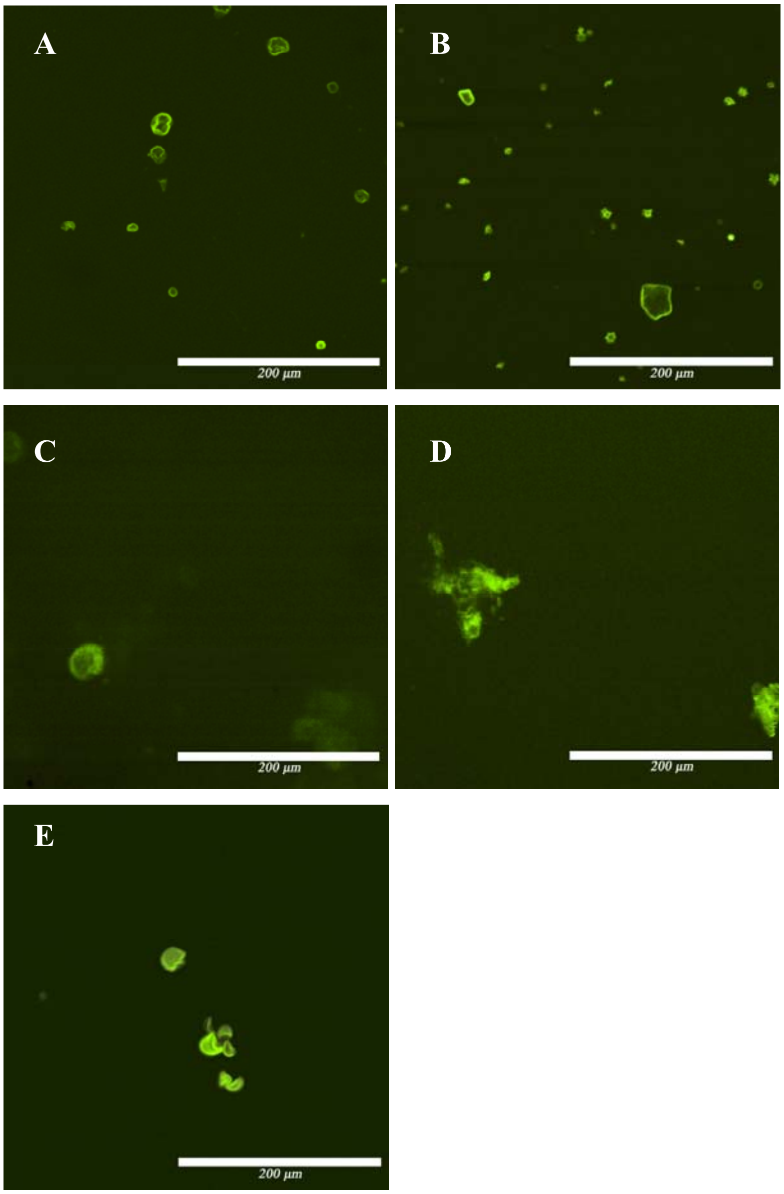

Figure 4. Laser scanning confocal micrograph of fluorescently labeled (A) waxy corn starch, (B) waxy rice starch, (C) instant tapioca starch, (D) modified tapioca starch, and (E) dextrin.

These starches are known to be smaller and more soluble than WC, WR, and IT, and the several washing steps required to properly gelatinize, oxidize and stain the starches may have removed much of the starch from the solutions. There was also a general background staining in all starch gels imaged because it is difficult to remove all excess acriflavine from the solutions. However, the amount of fluorescence is greater in areas where the dye is concentrated and since acriflavine binds covalently to the starch there is more fluorescence in areas where there are more starch aldehyde groups per unit volume. When staining starch within skim milk gels, there are fewer washing steps involved and the starch remains entrapped in the protein network, so washing away soluble starch materials present in the protein gel is not as much of a concern.

Microstructure and Starch Retention

LSCM method. Curd samples to be used for LSCM imaging were initially stored in an osmium tetroxide in whey solution. The purpose of this step was to fix the protein and make the sample firmer and therefore easier to work with. Previous attempts to work with the curd in its original state were unsuccessful as the curds could not be thinly sliced, were too soft, and fell apart during washing and staining steps. Osmium was selected as the fixative of choice because of its ability to crosslink proteins without changing the microstructure of the material (Bourne, 1967). Also, unlike glutaraldehyde, it did not add free aldehyde groups to the sample, which was important because of the starch-staining technique. Controlled microwaving of the samples during fixing, staining, and washing steps was done to hasten penetration of the fixative, stain, etc. (Horobin and Flemming, 1990).

Since acriflavine fluoresces without being bound to starch, a general background fluorescence from acriflavine is expected in the images obtained with LSCM. However, most excess acriflavine can be rinsed from the sample, and since the dye is concentrated in starch-rich areas and degree of fluorescence is related to stain concentration and localization, starch identification in the curd is possible. Rhodamine B was used to stain the protein. Since skim milk was used to make the gel, the amount of fat present in the curd was minimal and was not imaged. Also, a stain for fat that fluoresced at different wavelengths than the acriflavine and rhodamine B was not available. Thus, it was assumed any fat present behaved similarly in all samples and was not affected by starch addition.

In LSCM images where the protein network appeared mostly continuous with starch interspersed throughout, the network was considered disrupted. In those where the protein was less continuous, the network was described as loose. If the protein network dominated the structure with few interruptions or voids, the network was considered dense or homogenous.

Control gel. When skim milk gels made without starch addition were made using either the 72°C or 66°C for 30 min heat treatments, there were no differences in microstructure, curd and whey yields, or alcohol-insoluble residue in whey. So for the remainder of this chapter, control gel will refer to gels made with the 72°C heat treatment. Gels made with no starch had a microstructure that would be expected in a low fat or non-fat system The protein matrix dominated the structure, with few interruptions (Figure 5). Following the centrifugation step, the gel was visibly more compact than gels containing starch, and the curd yield of 13.1 g curd per 100 g milk was significantly

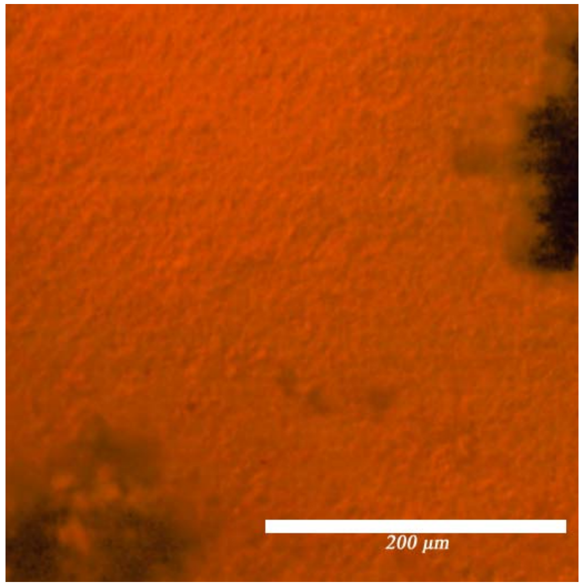

Figure 5. Control skim milk gels as observed with LSCM. Red-orange represents protein, dark areas have no fluorescence and represent serum voids containing whey.

lower than most starch-containing gels. The amount of residue recovered after alcohol precipitation and filtration of control whey was only 1.79 mg/g whey and is likely comprised of proteins.

Waxy corn starch. Addition of WC starch to skim milk prior to gelation significantly increased the curd yield to 18.4 g curd per 100 g milk (Table 4), and the openness of the gel's structure was evidence of the increased volume (Figure 6). The gel structure is more open than the control gel with the protein network less compact and swollen starch particles up to about 50 µm in size were distributed throughout the matrix. The swollen starch particles were relatively large, and irregularly shaped, and the granules appear to still remain somewhat intact. The starch particles appear to be interacting with protein strands' surfaces, rather than being suspended in the moisture rich areas. An increased amount of dark space in the micrographs indicates more moisture was incorporated into the curd. Moisture rich areas also likely contain soluble starch, but at a level where it cannot be distinguished from background staining in the

Table 4. Curd and whey yields after centrifugation of acidified renneted milk containing 5 g/L starch, along with alcohol-insoluble residue in the whey and calculated starch content of the whey and retention in the curd.

	Control	WC Starch	WR Starch	IT Starch	MT Starch	D Starch
Curd yield, g/100 g milk	13.1[a]	18.4[b]	20.7[c]	21.5[c]	23.5[d]	13.2[a]
Alcohol insoluble residue, mg/g whey	1.79 [a]	3.92 [abc]	3.24 [ab]	2.38 [a]	6.30 [bc]	7.09 [c]
Total starch in whey[2], g	n/a[1]	0.39[ab]	0.26[a]	0.10[a]	0.79[bc]	0.98[c]
Starch Retention in Curd[3], %	n/a	71[ab]	90[a]	90[a]	21[bc]	1[c]

[abc] means within a row with the same letter were not different, $\alpha = 0.05$
[1] not applicable, no starch added
[2] calculated as amount of alcohol insoluble residue from whey less amount of residue from control whey, then multiplied by the amount of whey recovered from starch-containing curds after centrifugation.
[3] calculated as amount added to milk less amount lost in whey

images. The residue recovered from whey expelled from the WC starch gel was 3.92 mg/g, which implies 71% retention of starch within the curd. This is consistent with the structure of the material, as a large volume of the curd structure is filled with starch particles.

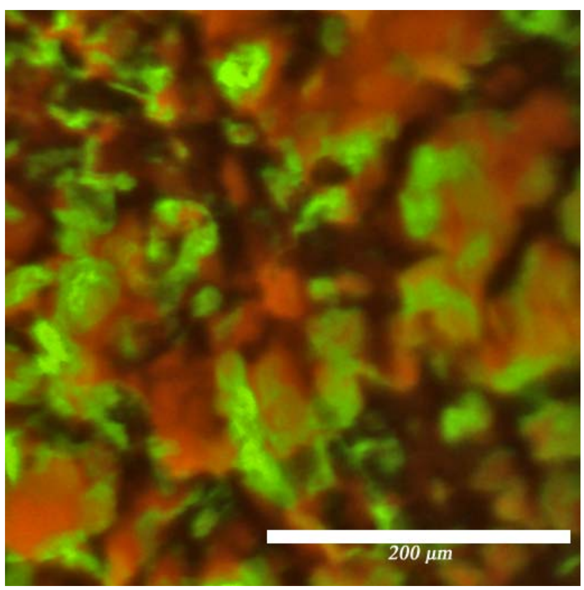

Figure 6. Skim milk gel containing waxy corn starch as observed with LSCM. Red-orange represents protein, green represents starch, and dark areas represent serum voids containing whey

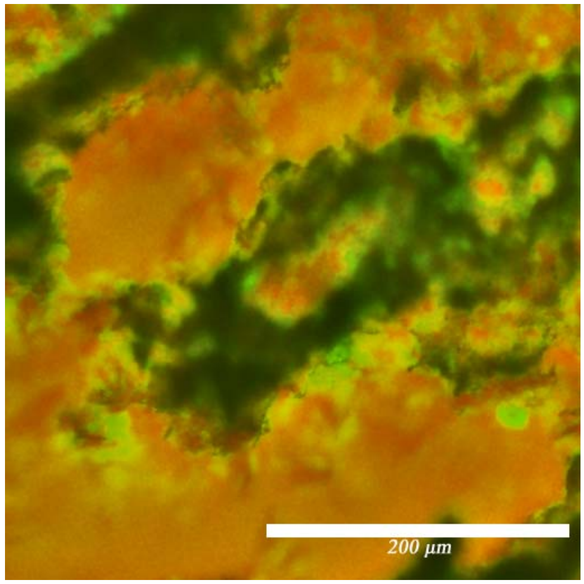

Figure 7. Skim milk gel containing WR starch as observed with LSCM. Red-orange represents protein, green-yellow represents starch, and dark areas represent serum voids containing whey.

Waxy rice starch. The milk gel made with WR starch also had a more open structure and significantly higher curd yield than the control. The curd yield was 20.7 g/100 g milk (Table 4). The structure was a loose protein network extensively coated in small, round, discrete starch moieties (Figure 7). Starch was located primarily at the protein-void interfaces, but some particles were visibly trapped within the protein network as well. The swollen starch granules were small and typically < 10 μm. From the curd structure it is clear that a high amount of starch was retained with the curd. The whey had 3.24 mg residue per gram of whey, meaning approximately 90% of the starch added to the skim milk was retained in the curd. The curd made with WR starch had large areas of intact protein (Figure 7) whereas curd with WC starch (Figure 6) had a more interrupted protein network with smaller intact protein units. Waxy rice starch particles were smaller than WC, and there was a considerable amount of WR starch in the serum voids, unlike in the WC curd where WC appears more discrete in nature.

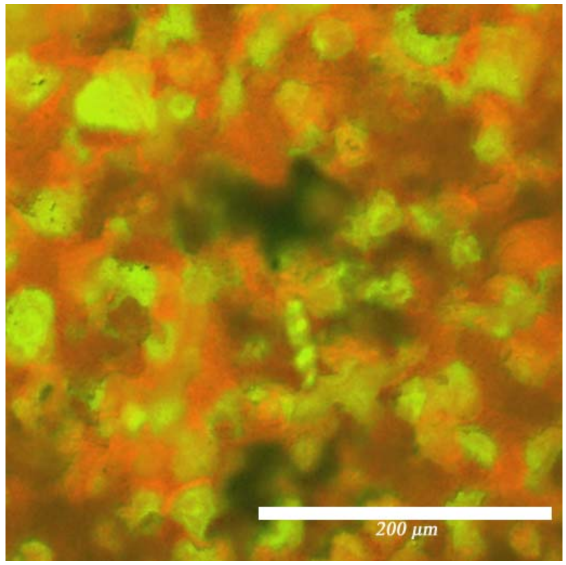

Figure 8. Skim milk gels containing IT starch as observed with LCSM. Red-orange represents protein, green-yellow represents starch, and dark areas represent serum voids containing whey.

Instant tapioca starch. Inclusion of IT starch in the skim milk gels also resulted in high curd yields, and a more disrupted curd structure (Figure 8). The amount of residue per gram of whey was 2.38 mg, which was not statistically different from the control, indicating good starch retention in the curd. With a curd yield of 21.5 g/100 g milk, and an estimated starch retention of 90%, addition of IT starch to the milk resulted in the curd with nearly the highest yield and the highest amount of starch retention (Table 4). Unlike the WC and WR starches, IT starch did not appear to be preferentially located at the protein-void interfaces. Rather, it was dispersed throughout the protein matrix and filled the spaces it created in the protein, much as fat globules in full fat cheese do. The swollen starch particles were irregularly shaped and evenly distributed through the protein matrix.

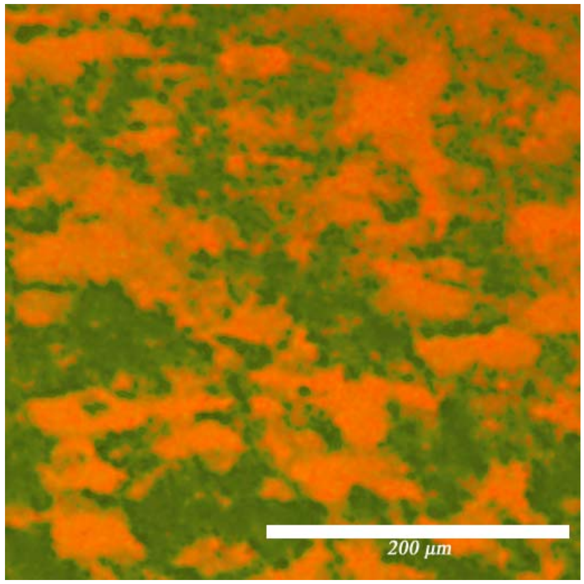

Figure 9. Skim milk gels containing MT starch as observed with LSCM. Red-orange represents protein, green-yellow represents starch, and dark areas represent serum voids containing whey.

Modified tapioca starch. The milk gel made with MT produced a curd structure with the most interruptions. The protein network appeared very loose, with numerous voids, although there were few distinct starch structures (Figure 9). The amount of residue per gram of whey was significantly higher than the control for the whey made with the modified tapioca starch, indicating poor starch retention in the curd (Table 4). The estimated starch retention was 21%, and based on the lack of distinct starch regions in the curd structure the estimate seems correct. However, the modified tapioca starch very effectively disrupted the protein network creating large channels. Incorporation of high amounts of moisture into those channels helped increased the curd yield to 23.5 g /100 g milk – the highest yield of all the starch-containing gels. In solution with just water, the modified food starch has a high swelling power, and the slimy, mucous-like gel it creates does not sediment when centrifuged at 2,000 *g* for 20 min. Likely the starch interacted highly with the protein, inhibiting some of the protein-protein interactions that occur when milk is renneted and acidified, but due to the highly soluble nature of the starch, a large portion was lost with the whey and only the portion that was physically entrapped or interacting the with protein remained in the curd.

Dextrin. Addition of D starch did not increase openness of the skim milk gels (Figure 10), nor did it significantly increase the curd yield over the control curd (Table 4). The D starch curd yield was only 13.2 g / 100 g of milk, and the estimated starch retention was only 1%. The structure of the curd is similar to the control as it is dominated by protein, with few disruptions. There are some small, distinct starch particles imbedded in the protein that are likely some residual granules from preparation of the dextrin powder and are there only because they were physically entrapped in the curd as the curd formed and was compressed. Due to the low retention of dextrin in the curd and its inability to change the curd structure, it is likely non-interacting with the caseins.

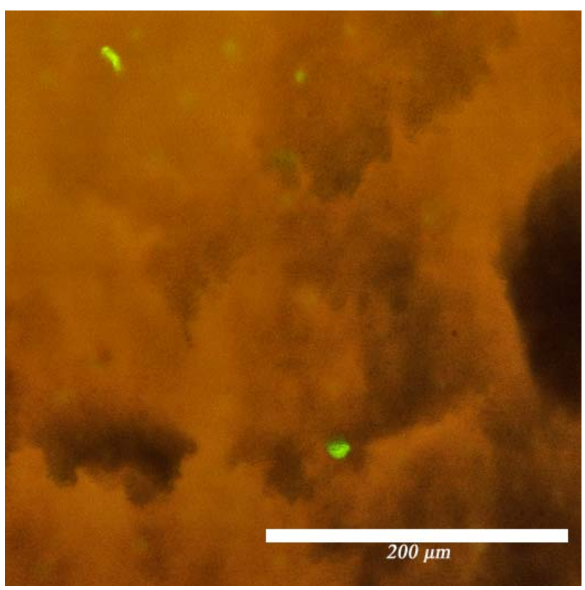

Figure 10. Skim milk gel containing D as observed with LSCM. Red-orange represents protein, green-yellow represents starch, and dark areas represent serum voids containing whey.

DISCUSSION

Typically cheddar cheese yield is about 9 to 10%, and the yield for the control curd in this model system was 13%, which would be expected as the model system leaves out some cheesemaking steps that further concentrate curd components. Control cheeses for the two heat treatments were nearly identical. Both were very firm and rubbery, and had yields at about 13%. They were difficult to cut through, and had very good curd and whey separation.

Waxy corn starch has a high amylopectin content, and because it is crosslinked and modified it is more resistant to degradation than its native counterpart. At 72°C, WC has a high swelling power relative to lower temperatures, but likely swells more if given a more severe heat treatment. Because it is more resistant, some granules are likely still intact within the curd matrix. In imitation low fat cheese made with resistant corn starch, the starch was shown to be granular still and dispersed homogenously throughout the matrix, rather than forming swollen clusters (Noronha et al., 2007). Curd yield was close to 19%, evidence of WC's high affinity for water combined with prevention of curd shrinkage during centrifugation

Waxy rice starch was retained well in the curd and also increased yield more than other starches with the 72°C heat treatment. WR starch has very small granules, high water binding capacity, and high amylopectin content. Because the unswollen granules only range from 3-8 um, they can impart a creamy mouthfeel to the products in which they are used. Rice starches have often been used as a fat mimetic. In the skim milk gel with WR starch there were good retention in the curd, and also a high yield. Curd was soft and voluminous, similar to the curd made with the WC starch. In a study of acidified skim milk containing rice starch (Zuo et al., 2007), it was found that rice starch acts as an inactive filler and absorbs water from the continuous phase thereby concentrating the protein, a trait likely more desirable in acidified milk products like yogurt where whey expulsion is undesirable, but in products like low fat cheese further protein concentration is unwanted. If the heat treatment given to the milk and starch prior to cheese making is sufficient to cause maximal granule swelling prior to renneting and acidification of the milk, it may be possible to inhibit further water uptake by the starch from the protein.

Instant tapioca starch had very good retention in the curd. This cheese had soft, cohesive curd, and good curd and whey separation. Low- and reduced-fat feta cheese with tapioca starch has been studied (Sipahioglu et al., 1999), and it was found that tapioca starch increased moisture and hardness of cheese, and did not significantly improve texture, flavor or overall acceptability over low fat controls. In that study, though, milk did not receive an appreciable heat treatment once starch was added, suggesting the starch was more likely to absorb water from the protein during storage, and it may have given a grainy feel to the cheese. In our study instant tapioca starch was used, which requires very little heat for dispersion and swelling to occur

The MT starch is a starch typically used to change the mouth feel in dairy products. In the skim milk gels, it made the curd very soft, and not as cohesive as the curds made with the other starches. Boundaries where the curd was cut didn't meld back together well, as they did in other cheeses. The whey decanted from this cheese was thicker and slimier than control whey, indicating starch loss in the whey. This was confirmed by the whey analysis test which showed only 21% retention in the curd. Interestingly, this cheese also had the highest yield, perhaps indicative of to the high hygroscopicity of this starch. The MT starch disrupts some of the protein interactions that normally would occur in renneted, acidified milk that would also restrict curd shrinkage and syneresis. This trait was even more apparent in cheeses made with 10 g/L starch in milk (data not shown), where separation of curd and whey was nearly impossible. However, at 5 g/L, this starch could likely impart the desired discontinuity to low fat cheese by interfering with the hydrophobic interactions that hold the protein matrix together.

All cheeses made with starch had higher yields than controls, except for the D starch-containing cheese. This was expected because in tests measuring the swelling power of the starches with the heat treatments used, the dextrin had a very low swelling power (data not shown). The D starch had the lowest retention in the curd, probably because it is made up of smaller molecules that are less likely to get trapped within the curd. Dextrins are also more soluble than higher molecular weight starches making them more likely to remain in the whey. The curd made with D starch had an appearance and feel similar to control, which was firm and rubbery. Although inclusion of the D starch didn't greatly enhance the yield, its low swelling ability could be desirable in cheese.

CONCLUSION

Low fat cheese curd normally has a dense protein network. Addition of WC starch produced a loose protein network with irregularly shaped starch granules evenly distributed around and throughout that matrix; retention of WC in the curd was 71%. Waxy rice starch had 90% retention in the curd. Waxy rice starch curd was also less dense, but the small granules of starch coated the proteins strands rather than being distributed throughout the curd. Instant tapioca starch similarly coated protein strands but was not in granular form. It was also retained well in the curd with a retention of 90%. The MT starch interacted highly with the protein, as the curd network was very loose. The starch seems to be in solution around, as well as incorporated into the protein, but the starch was only 12% retained. Dextrin starch interacted little with the protein, resulting in only 1% starch retention and a dense protein network with little starch entrapment in the curd. All starches except D increased the curd yield with the effect being MT > IT=WR > WC > D=control.

Waxy corn, WR, and IT starches have the potential to improve the texture of low fat cheese because they are retained well in the protein network during coagulation and concentration of the milk proteins, and they generate interruptions in curd network that may help limit extensive protein-protein interactions. Modified tapioca starch caused the protein structure of the curd to be very loose, but it was retained poorly in the curd. Also, because there were few distinct starch particles in the MT curd network, it is likely that when it is subjected to all the cheesemaking steps the same loose protein structure would not be observed. Dextrin was not retained well in the curd, nor did it disrupt the protein network, making it unsuitable for use in low fat cheese.

CHAPTER 5

EFFECT OF STARCH ADDITION ON LOW FAT CHEDDAR CHEESE

INTRODUCTION

To test the suitability of using starches in manufacturing low fat Cheddar cheese, some exploratory trials were conducted in which Cheddar cheese was made with added starch. Starches were selected based on data obtained from Chapters 3 and 4. The aim was to determine if addition of hydrated starch to low fat cheese would increase moisture content, yield, and provide desirable discontinuities within the protein network.

MATERIALS AND METHODS

Starches

The starches used were modified waxy cornstarch (WC) (starch 1 in Chapter 3), waxy rice starch (WR) (starch 7 in Chapter 3), instant tapioca starch (IT) (starch 9 in Chapter 3), and a modified tapioca starch (MT) (starch 10 in Chapter 3), all obtained from National Starch Food Innovation (Bridgewater, NJ). Starches were added to water at 7% (wt/vol), then pregelatinized with a heat treatment of either 72°C (then immediately cooled) for the WC and WR starch or 66°C for 30 minutes for the IT and MT starches. Starch solutions were heated in a conventional microwave using 1 and 2 min heating increments at high power until temperature was reached. Starches receiving the 66°C heat treatment were then held in a 68°C waterbath to maintain the 66°C solution temperature for 30 min.

Cheese Making

Milk with 0.6% fat from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan, UT) was pasteurized at 73°C for 15 s, and cooled to 4°C. Prior to cheesemaking, milk was prewarmed to 55°C, then cooled to 31°C. A control batch of low fat Cheddar cheese without starch and three batches containing starches were made using 15.9 kg of milk per batch. Milk was inoculated with 7 g of DVS 850 starter culture containing a mixture of *Lactococcus lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis* (Chr. Hansen, Inc., Milwaukee, WI). After 20 min, 1.8 ml of single strength annatto (Maxiren DS, DSM Food Specialties, Parsippany, NJ) was added to each vat, and after and additional 35 min of ripening, 1.3 kg of 7% (wt/vol), starch slurries were stirred into the designated batches. After 40 min, 3.5 ml of double strength chymosin (Maxiren DS, DSM Food Specialties) diluted to 35 ml were added to each vat of milk and a firm set was attained in 20 min. Curd was cut with 6.4-mm wire knives horizontally and vertically and allowed to heal for 5 min. Curd was stirred gently for 23 min, then stirred more as temperature was raised from 31°C to 36°C over 15 min. Once curd pH reached 6.2, it was drained, and then washed with 1.8 kg cold water to cool the curd to 27°C. Curd was drained again, and then stirred till it reached pH 5.85, at which point it was salted at a rate of 26 g/kg curd for starch-containing curd and 22 g/kg curd for control curd. Curd was hooped, pressed overnight at 55 kPa, de-hooped then vacuum-sealed and stored at 4°C.

The first trial of cheesemaking was just exploratory and in subsequent trials the make procedure was modified based on results from previous trials to make a more satisfactory cheese. The first trial of cheesemaking used MT, IT and WC starches. The other trials used IT, WC, and WR starches. The MT starch did not change the texture or volume of the cheese as compared to control when it was used in the first trial, so it was not examined in further trials.

For the second trial, cheeses were made as previously described, except the washing and second drain step were excluded. Curd was initially drained at 6.2, then dry stirred till 5.85 at which point it was salted.

For the third trial, the cheese make procedure also lacked the washing and second drain step. Additionally, the curd was cooked from 31°C to 39°C over 30 minutes, and the drain pH was raised to 6.3, and vats were inoculated with only 3.5 g of the DVS 850 culture in order to slow acidification, half of what was previously used.

Compositional Analysis

All cheeses were tested for pH, salt content, fat content and moisture content after 1 week. Fat was measured by the Babcock method. Moisture was measured by vacuum oven drying, pH with a glass electrode, and salt was measured with a Corning chloride analyzer.

RESULTS

Cheeses made according to the first procedure were too soft and did not have desirable textures according to visual observation. When cheeses were removed from the hoops after pressing, the blocks containing WC and IT starches resembled full fat cheese in appearance and apparent firmness. However, during cold storage the cheeses softened considerably and no longer retained their original cylindrical shape. The body of the cheeses became very pasty and undesirable.

Table 5. Compositions of starch-added low fat Cheddar cheeses

Trial	Treatment	Moisture (%)	Fat (%)	Salt (%)	pH	Block weight (kg)
1	Control	50.24	6.5	1.89	5.22	1.09
	WC	65.28	3.5	2.18	4.93	1.82
	IT	60.35	5.0	2.07	5.02	1.54
	MT	51.35	5.5	1.8	5.26	1.11
2	Control	50.75	5	1.82	5.15	1.00
	WC	63.23	3.0	2.34	5.15	1.63
	IT	64.11	2.5	2.26	5.23	1.68
	WR	63.52	3.0	2.33	5.08	1.63
3	Control	47.55	7.0	2.0	5.37	1.02
	WC	62.14	5.0	2.28	5.21	1.50
	IT	60.66	4.0	2.06	5.08	1.32
	WR	57.40	5.0	2.10	5.22	1.32

The moisture contents of the WC and IT starch cheeses were very high, contributing to the excessive softness of the blocks (Table 5). The cheese made with MT starch resembled the control that contained no starch in its appearance and its texture, according to visual observations and touch. The texture of the cheese did not visibly soften during storage though, as it did with the other starch-containing cheeses.

Since the MT starch did not seem to improve the texture of low fat cheese, it was not examined in further experiments. For the second trial of cheese making, WC, IT, and WR starches were used. The procedure used in the first trial was followed for the second round, except the washing and second draining steps were omitted to try to decrease the moisture content of the starch-containing cheeses. The resultant blocks, however, still contained too much moisture. As in the first trial, the blocks containing starch softened excessively during storage.

In the third trial, changes were made to the make procedure to reduce the moisture content of the cheeses to try to limit the excessive softening that appeared to occur. The moisture content of the cheeses was reduced, but they knitted very poorly. We were going to perform textural analysis using a Texture Profile Analyzer (TPA) to characterize the softing over time patter that we observed in previous trials, but we were unable to get good samples for analysis because when the cheeses were cut they would crumble apart. We tried to get samples after 1 d and several times over the first 2 weeks, but the starch-containing blocks never knitted properly in that time to get decent samples.

DISCUSSION

During cold storage of the cheese blocks from trials 1 to 3, those made with WC, WR, or IT starches underwent significant visible softening. After a couple days, blocks no longer retained their cylindrical shape. When blocks were initially removed from the press, they felt much firmer than after cold storage. It is possible that during storage, the starch and protein demix, separating into protein rich areas and starch rich areas. Once the starch is concentrated, the molecules of amylose and amylopectin begin to reassociate forming crystalline starch regions exclusive of water. Since the starch molecules that have recrystallized no longer hold water, the water that was once bound then becomes free water which can diffuse into the protein rich areas thus softening the cheese. Koca and Metin (2003), observed a similar effect when Simplesse was added to low fat Kashar cheese. The cheese was rated as more acceptable than the control up until 30 d of storage, but with longer storage time acceptability ratings decreased because excessive softening occurred.

With the cheeses made from trial 4, however, after 3 w the curd particles had not knitted together, so the block was very crumbly when cut. The moisture contents of these blocks were lower than for other reps. Likely the amount of water available for protein hydration and knitting was not sufficient. Retrogradation of the starches and subsequent release of water also did not occur. In order for the starch molecules to associate enough to form crystalline regions they need to have mobility, and by lowering the moisture content of the cheese that mobility is also limited.

CONCLUSION

I was not able to satisfactorily manufacture low fat Cheddar cheese containing starches. The cheeses with starch were too high in moisture and too soft, especially after cold storage, which may have allowed the starches to retrograde, and when the moisture content was lowered the curd did not knit together properly. These problems could potentially be eliminated by reducing the amount of starch added, using different starches, or adding other ingredients such as emulsifiers to limit starch retrogradation.

CHAPTER 6

GENERAL SUMMARY

Seventeen starches were first screened to determine their influences on renneted, acidified skim milk curd yields and also characterize their swelling properties. From these starches, 5 were selected to further investigate in the skim milk gel system. The 5 starches examined were waxy corn starch, waxy rice starch, instant tapioca starch, modified tapioca starch, and dextrin. Determining the extent of incorporation of these starches into acidified rennet-induced skim milk gels, and starch impact on the microstructure of these gels were the primary goals of the investigation.

Each starch was first added to skim milk and then given a heat treatment to gelatinize the starch. The milk solutions were next renneted and acidified with GDL, incubated to allow coagulum formation, then centrifuged to sediment curd from whey. They whey from each sample was then analyzed for starch content to determine the starch retention in the curd. Curds yields were 13.1%, 18.4%, 20.7%, 21.5%, 23.5%, and 13.2% for control gel, and gels containing waxy corn, waxy rice, instant tapioca, modified tapioca and dextrin starches, respectively. Estimated starch retentions in the curds were 71%, 90%, 90%, 21%, and 1% for these curds.

Using LSCM we were able to see the impact of the starches on skim milk curd microstructure. Low fat cheese curd normally has a dense protein network. Addition of waxy corn starch produced a loose protein network with irregularly shaped starch granules evenly distributed around and throughout that matrix. Waxy rice starch curd was also less dense, but the small granules of starch coated the proteins strands rather than being distributed throughout the curd. Instant tapioca starch similarly coated protein strands but was not in granular form. The modified tapioca starch interacted highly with the protein, as the curd network was very loose. The starch seems to be in solution around, as well as incorporated into the protein. Dextrin interacted little with the protein, resulting in a dense protein network with little starch entrapment in the curd.

Since dextrin was not retained in the skim milk gel and it did not affect the curd texture, it was not examined for use in low fat Cheddar cheese. Waxy corn, waxy rice, instant tapioca, and modified tapioca starches were selected for testing in low fat Cheddar cheese because they all increased curd yield of the skim milk gels and disrupted the curd protein network. Waxy corn, waxy rice, and instant tapioca also were retained well in the skim milk gels, a desirable trait since it would minimize losses in the whey and the amount of starch necessary to achieve the desired effect. Although the modified tapioca starch was not retained as optimally in the skim milk gel curd, because of its ability to cause such a loose protein network it was also selected.

The low fat Cheddar cheese with modified tapioca starch did not have a higher yield than the control low fat cheese containing no starch so its use in cheese-making was limited to the first trial only. The other starches did increase the yield over the control, and increased the moisture content of the cheese by 10% to 15%. However, during cold storage of cheese blocks from the first 3 trials of cheesemaking, the blocks underwent excessive softening due to starch retrogradation and subsequent release of moisture. The starch-containing cheeses from trial 4, despite having moisture contents between 57% and 62%, did not knit together well and crumbled apart upon cutting the blocks.

Although we were unable to successfully make acceptable low fat Cheddar cheese containing starch, there is still potential for the idea to work. Future research on modifications of cheesemaking procedures for low fat cheese with added starch, addition of other ingredients such as emulsifiers to the starch prior to cheesemaking, use of less starch or other starches, or novel manufacturing technologies would all aid in the successful development of a low fat Cheddar cheese. We were able to show that starches can be retained well in curd, and that starches can disrupt some of the protein-protein interactions that contribute to the over-firm, rubbery texture of low fat cheese. Starch also can increase the moisture content of low fat Cheddar, but there are problems with excess softening during storage and poor curd knitting.

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APPENDICES

APPENDIX A

CALCULATIONS

Calculations for Curd and Whey Yield

$$Y = C \div M \times 100 \qquad X = W \div M \times 100$$

Curd weight = C

Total Milk Weight = M

Whey Weight = W

Curd Yield = Y

Whey Yeild = X

Calculation for whey analysis recovery factors

$$R \div K = C$$

Known weight of starch in analysis = K

Recovered weight of starch in analysis = R

Recovery Factor = C

Calculation for Estimated Percent Starch Retention in the Curd

$$P = 100 - \{ ([(S - C) \times W] \div T) \times 100 \}$$

Residue per gram of starch-containing whey = S

Residue per gram of control whey = C

Weight of total whey decanted following centrifugation of starch milk gel = W

Total amount of starch added to milk = T

Estimated Percent starch retention in the curd = P

Approximate weight of starch in total whey = $(S - C) \times W$

APPENDIX B

STATISTICAL TABLES

LSMEAN for milks with 5 g/L starch. Treatment 1: 63; Treatment 2: 63 held for 30 min; Treatment 3: 72 . Starch numbers reference Table 1. Starch 0 means control- no starch added

treatment	starch	curd LSMEAN	LSMEAN Number
1	0	11.8000000	1
1	1	15.2500000	2
1	2	18.2500000	3
1	3	19.5000000	4
1	4	17.2500000	5
1	5	21.5000000	6
1	6	13.5000000	7
1	7	12.7500000	8
1	8	19.7500000	9
1	9	22.0000000	10
1	10	23.5000000	11
1	11	12.2500000	12
1	12	11.0000000	13
1	13	11.7500000	14
1	14	11.2500000	15
1	15	16.0000000	16
1	16	16.7500000	17
1	17	18.0000000	18
2	0	12.4166667	19
2	1	17.5000000	20
2	2	20.2500000	21
2	3	20.7500000	22
2	4	18.2500000	23
2	5	23.5000000	24
2	6	14.2500000	25
2	7	13.2500000	26
2	8	18.7500000	27
2	9	18.5000000	28

treatment	starch	curd LSMEAN	LSMEAN Number
2	**10**	21.7500000	29
2	**11**	11.7500000	30
2	**12**	11.0000000	31
2	**13**	12.7500000	32
2	**14**	12.0000000	33
2	**15**	18.2500000	34
2	**16**	17.7500000	35
2	**17**	19.7500000	36
3	**0**	13.6000000	37
3	**1**	20.0000000	38
3	**2**	21.2500000	39
3	**3**	25.0000000	40
3	**4**	17.5000000	41
3	**5**	25.0000000	42
3	**6**	15.7500000	43
3	**7**	21.2500000	44
3	**8**	20.2500000	45
3	**9**	20.0000000	46
3	**10**	28.0000000	47
3	**11**	12.5000000	48
3	**12**	14.2500000	49
3	**13**	15.7500000	50
3	**14**	12.5000000	51
3	**15**	18.0000000	52
3	**16**	28.5000000	53
3	**17**	21.0000000	54

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.2329	<.0001	<.0001	0.0003	<.0001	0.9998	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
2	0.2329		0.8820	0.1819	0.9999	0.0008	1.0000	0.9893	0.1064	0.0001	<.0001	0.8820	0.1819	0.5991
3	<.0001	0.8820		1.0000	1.0000	0.7576	0.0589	0.0077	1.0000	0.4352	0.0157	0.0017	<.0001	0.0003
4	<.0001	0.1819	1.0000		0.9984	0.9999	0.0017	0.0001	1.0000	0.9893	0.2917	<.0001	<.0001	<.0001
5	0.0003	0.9999	1.0000	0.9984		0.1819	0.4352	0.1064	0.9893	0.0589	0.0008	0.0311	0.0008	0.0077
6	<.0001	0.0008	0.7576	0.9999	0.1819		<.0001	<.0001	1.0000	1.0000	0.9999	<.0001	<.0001	<.0001
7	0.9998	1.0000	0.0589	0.0017	0.4352	<.0001		1.0000	0.0008	<.0001	<.0001	1.0000	0.9893	1.0000
8	1.0000	0.9893	0.0077	0.0001	0.1064	<.0001	1.0000		<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
9	<.0001	0.1064	1.0000	1.0000	0.9893	1.0000	0.0008	<.0001		0.9984	0.4352	<.0001	<.0001	<.0001
10	<.0001	0.0001	0.4352	0.9893	0.0589	1.0000	<.0001	<.0001	0.9984		1.0000	<.0001	<.0001	<.0001
11	<.0001	<.0001	0.0157	0.2917	0.0008	0.9999	<.0001	<.0001	0.4352	1.0000		<.0001	<.0001	<.0001
12	1.0000	0.8820	0.0017	<.0001	0.0311	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001		1.0000	1.0000
13	1.0000	0.1819	<.0001	<.0001	0.0008	<.0001	0.9893	1.0000	<.0001	<.0001	<.0001	1.0000		1.0000
14	1.0000	0.5991	0.0003	<.0001	0.0077	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	
15	1.0000	0.2917	<.0001	<.0001	0.0017	<.0001	0.9984	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
16	0.0295	1.0000	0.9984	0.5991	1.0000	0.0077	0.9893	0.7576	0.4352	0.0017	<.0001	0.4352	0.0311	0.1819
17	0.0022	1.0000	1.0000	0.9569	1.0000	0.0589	0.7576	0.2917	0.8820	0.0157	0.0001	0.1064	0.0037	0.0311
18	<.0001	0.9569	1.0000	1.0000	1.0000	0.5991	0.1064	0.0157	1.0000	0.2917	0.0077	0.0037	<.0001	0.0008
19	1.0000	0.6190	<.0001	<.0001	0.0022	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
20	0.0001	0.9984	1.0000	0.9999	1.0000	0.2917	0.2917	0.0589	0.9984	0.1064	0.0017	0.0157	0.0003	0.0037
21	<.0001	0.0311	0.9999	1.0000	0.8820	1.0000	0.0001	<.0001	1.0000	1.0000	0.7576	<.0001	<.0001	<.0001
22	<.0001	0.0077	0.9893	1.0000	0.5991	1.0000	<.0001	<.0001	1.0000	1.0000	0.9569	<.0001	<.0001	<.0001
23	<.0001	0.8820	1.0000	1.0000	1.0000	0.7576	0.0589	0.0077	1.0000	0.4352	0.0157	0.0017	<.0001	0.0003
24	<.0001	<.0001	0.0157	0.2917	0.0008	0.9999	<.0001	<.0001	0.4352	1.0000	1.0000	<.0001	<.0001	<.0001
25	0.9086	1.0000	0.2917	0.0157	0.8820	<.0001	1.0000	1.0000	0.0077	<.0001	<.0001	0.9999	0.7576	0.9893
26	1.0000	0.9999	0.0311	0.0008	0.2917	<.0001	1.0000	1.0000	0.0003	<.0001	<.0001	1.0000	0.9984	1.0000
27	<.0001	0.5991	1.0000	1.0000	1.0000	0.9569	0.0157	0.0017	1.0000	0.7576	0.0589	0.0003	<.0001	<.0001
28	<.0001	0.7576	1.0000	1.0000	1.0000	0.8820	0.0311	0.0037	1.0000	0.5991	0.0311	0.0008	<.0001	0.0001
29	<.0001	0.0003	0.5991	0.9984	0.1064	1.0000	<.0001	<.0001	0.9999	1.0000	1.0000	<.0001	<.0001	<.0001
30	1.0000	0.5991	0.0003	<.0001	0.0077	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
31	1.0000	0.1819	<.0001	<.0001	0.0008	<.0001	0.9893	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
32	1.0000	0.9893	0.0077	0.0001	0.1064	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
33	1.0000	0.7576	0.0008	<.0001	0.0157	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
34	<.0001	0.8820	1.0000	1.0000	1.0000	0.7576	0.0589	0.0077	1.0000	0.4352	0.0157	0.0017	<.0001	0.0003
35	<.0001	0.9893	1.0000	1.0000	1.0000	0.4352	0.1819	0.0311	0.9999	0.1819	0.0037	0.0077	0.0001	0.0017
36	<.0001	0.1064	1.0000	1.0000	0.9893	1.0000	0.0008	<.0001	1.0000	0.9984	0.4352	<.0001	<.0001	<.0001
37	0.9340	0.9999	0.0065	<.0001	0.1439	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	0.8341	0.9989
38	<.0001	0.0589	1.0000	1.0000	0.9569	1.0000	0.0003	<.0001	1.0000	0.9999	0.5991	<.0001	<.0001	<.0001

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
39	<.0001	0.0017	0.8820	1.0000	0.2917	1.0000	<.0001	<.0001	1.0000	1.0000	0.9984	<.0001	<.0001	<.0001
40	<.0001	<.0001	0.0001	0.0077	<.0001	0.5991	<.0001	<.0001	0.0157	0.8820	1.0000	<.0001	<.0001	<.0001
41	0.0001	0.9984	1.0000	0.9999	1.0000	0.2917	0.2917	0.0589	0.9984	0.1064	0.0017	0.0157	0.0003	0.0037
42	<.0001	<.0001	0.0001	0.0077	<.0001	0.5991	<.0001	<.0001	0.0157	0.8820	1.0000	<.0001	<.0001	<.0001
43	0.0632	1.0000	0.9893	0.4352	1.0000	0.0037	0.9984	0.8820	0.2917	0.0008	<.0001	0.5991	0.0589	0.2917
44	<.0001	0.0017	0.8820	1.0000	0.2917	1.0000	<.0001	<.0001	1.0000	1.0000	0.9984	<.0001	<.0001	<.0001
45	<.0001	0.0311	0.9999	1.0000	0.8820	1.0000	0.0001	<.0001	1.0000	1.0000	0.7576	<.0001	<.0001	<.0001
46	<.0001	0.0589	1.0000	1.0000	0.9569	1.0000	0.0003	<.0001	1.0000	0.9999	0.5991	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	0.0017	0.1064	<.0001	<.0001	<.0001
48	1.0000	0.9569	0.0037	<.0001	0.0589	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
49	0.9086	1.0000	0.2917	0.0157	0.8820	<.0001	1.0000	1.0000	0.0077	<.0001	<.0001	0.9999	0.7576	0.9893
50	0.0632	1.0000	0.9893	0.4352	1.0000	0.0037	0.9984	0.8820	0.2917	0.0008	<.0001	0.5991	0.0589	0.2917
51	1.0000	0.9569	0.0037	<.0001	0.0589	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
52	<.0001	0.9569	1.0000	1.0000	1.0000	0.5991	0.1064	0.0157	1.0000	0.2917	0.0077	0.0037	<.0001	0.0008
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0311	<.0001	<.0001	<.0001
54	<.0001	0.0037	0.9569	1.0000	0.4352	1.0000	<.0001	<.0001	1.0000	1.0000	0.9893	<.0001	<.0001	<.0001

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
1	1.0000	0.0295	0.0022	<.0001	1.0000	0.0001	<.0001	<.0001	<.0001	<.0001	0.9086	1.0000	<.0001	<.0001
2	0.2917	1.0000	1.0000	0.9569	0.6190	0.9984	0.0311	0.0077	0.8820	<.0001	1.0000	0.9999	0.5991	0.7576
3	<.0001	0.9984	1.0000	1.0000	<.0001	1.0000	0.9999	0.9893	1.0000	0.0157	0.2917	0.0311	1.0000	1.0000
4	<.0001	0.5991	0.9569	1.0000	<.0001	0.9999	1.0000	1.0000	1.0000	0.2917	0.0157	0.0008	1.0000	1.0000
5	0.0017	1.0000	1.0000	1.0000	0.0022	1.0000	0.8820	0.5991	1.0000	0.0008	0.8820	0.2917	1.0000	1.0000
6	<.0001	0.0077	0.0589	0.5991	<.0001	0.2917	1.0000	1.0000	0.7576	0.9999	<.0001	<.0001	0.9569	0.8820
7	0.9984	0.9893	0.7576	0.1064	1.0000	0.2917	0.0001	<.0001	0.0589	<.0001	1.0000	1.0000	0.0157	0.0311
8	1.0000	0.7576	0.2917	0.0157	1.0000	0.0589	<.0001	<.0001	0.0077	<.0001	1.0000	1.0000	0.0017	0.0037
9	<.0001	0.4352	0.8820	1.0000	<.0001	0.9984	1.0000	1.0000	1.0000	0.4352	0.0077	0.0003	1.0000	1.0000
10	<.0001	0.0017	0.0157	0.2917	<.0001	0.1064	1.0000	1.0000	0.4352	1.0000	<.0001	<.0001	0.7576	0.5991
11	<.0001	<.0001	0.0001	0.0077	<.0001	0.0017	0.7576	0.9569	0.0157	1.0000	<.0001	<.0001	0.0589	0.0311
12	1.0000	0.4352	0.1064	0.0037	1.0000	0.0157	<.0001	<.0001	0.0017	<.0001	0.9999	1.0000	0.0003	0.0008
13	1.0000	0.0311	0.0037	<.0001	1.0000	0.0003	<.0001	<.0001	<.0001	<.0001	0.7576	0.9984	<.0001	<.0001
14	1.0000	0.1819	0.0311	0.0008	1.0000	0.0037	<.0001	<.0001	0.0003	<.0001	0.9893	1.0000	<.0001	0.0001
15		0.0589	0.0077	0.0001	1.0000	0.0008	<.0001	<.0001	<.0001	<.0001	0.8820	0.9999	<.0001	<.0001
16	0.0589		1.0000	0.9999	0.1360	1.0000	0.1819	0.0589	0.9984	<.0001	1.0000	0.9569	0.9569	0.9893
17	0.0077	1.0000		1.0000	0.0134	1.0000	0.5991	0.2917	1.0000	0.0001	0.9893	0.5991	0.9999	1.0000

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
18	0.0001	0.9999	1.0000		0.0001	1.0000	0.9984	0.9569	1.0000	0.0077	0.4352	0.0589	1.0000	1.0000
19	1.0000	0.1360	0.0134	0.0001		0.0008	<.0001	<.0001	<.0001	<.0001	0.9985	1.0000	<.0001	<.0001
20	0.0008	1.0000	1.0000	1.0000	0.0008		0.9569	0.7576	1.0000	0.0017	0.7576	0.1819	1.0000	1.0000
21	<.0001	0.1819	0.5991	0.9984	<.0001	0.9569		1.0000	0.9999	0.7576	0.0017	<.0001	1.0000	1.0000
22	<.0001	0.0589	0.2917	0.9569	<.0001	0.7576	1.0000		0.9893	0.9569	0.0003	<.0001	0.9999	0.9984
23	<.0001	0.9984	1.0000	1.0000	<.0001	1.0000	0.9999	0.9893		0.0157	0.2917	0.0311	1.0000	1.0000
24	<.0001	<.0001	0.0001	0.0077	<.0001	0.0017	0.7576	0.9569	0.0157		<.0001	<.0001	0.0589	0.0311
25	0.8820	1.0000	0.9893	0.4352	0.9985	0.7576	0.0017	0.0003	0.2917	<.0001		1.0000	0.1064	0.1819
26	0.9999	0.9569	0.5991	0.0589	1.0000	0.1819	<.0001	<.0001	0.0311	<.0001	1.0000		0.0077	0.0157
27	<.0001	0.9569	0.9999	1.0000	<.0001	1.0000	1.0000	0.9999	1.0000	0.0589	0.1064	0.0077		1.0000
28	<.0001	0.9893	1.0000	1.0000	<.0001	1.0000	1.0000	0.9984	1.0000	0.0311	0.1819	0.0157	1.0000	
29	<.0001	0.0037	0.0311	0.4352	<.0001	0.1819	1.0000	1.0000	0.5991	1.0000	<.0001	<.0001	0.8820	0.7576
30	1.0000	0.1819	0.0311	0.0008	1.0000	0.0037	<.0001	<.0001	0.0003	<.0001	0.9893	1.0000	<.0001	0.0001
31	1.0000	0.0311	0.0037	<.0001	1.0000	0.0003	<.0001	<.0001	<.0001	<.0001	0.7576	0.9984	<.0001	<.0001
32	1.0000	0.7576	0.2917	0.0157	1.0000	0.0589	<.0001	<.0001	0.0077	<.0001	1.0000	1.0000	0.0017	0.0037
33	1.0000	0.2917	0.0589	0.0017	1.0000	0.0077	<.0001	<.0001	0.0008	<.0001	0.9984	1.0000	0.0001	0.0003
34	<.0001	0.9984	1.0000	1.0000	<.0001	1.0000	0.9999	0.9893	1.0000	0.0157	0.2917	0.0311	1.0000	1.0000
35	0.0003	1.0000	1.0000	1.0000	0.0003	1.0000	0.9893	0.8820	1.0000	0.0037	0.5991	0.1064	1.0000	1.0000
36	<.0001	0.4352	0.8820	1.0000	<.0001	0.9984	1.0000	1.0000	1.0000	0.4352	0.0077	0.0003	1.0000	1.0000
37	0.9437	0.9276	0.4258	0.0153	0.9999	0.0730	<.0001	<.0001	0.0065	<.0001	1.0000	1.0000	0.0010	0.0026
38	<.0001	0.2917	0.7576	0.9999	<.0001	0.9893	1.0000	1.0000	1.0000	0.5991	0.0037	0.0001	1.0000	1.0000
39	<.0001	0.0157	0.1064	0.7576	<.0001	0.4352	1.0000	1.0000	0.8820	0.9984	<.0001	<.0001	0.9893	0.9569
40	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0589	0.1819	0.0001	1.0000	<.0001	<.0001	0.0008	0.0003
41	0.0008	1.0000	1.0000	1.0000	0.0008	1.0000	0.9569	0.7576	1.0000	0.0017	0.7576	0.1819	1.0000	1.0000
42	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0589	0.1819	0.0001	1.0000	<.0001	<.0001	0.0008	0.0003
43	0.1064	1.0000	1.0000	0.9984	0.2515	1.0000	0.1064	0.0311	0.9893	<.0001	1.0000	0.9893	0.8820	0.9569
44	<.0001	0.0157	0.1064	0.7576	<.0001	0.4352	1.0000	1.0000	0.8820	0.9984	<.0001	<.0001	0.9893	0.9569
45	<.0001	0.1819	0.5991	0.9984	<.0001	0.9569	1.0000	1.0000	0.9999	0.7576	0.0017	<.0001	1.0000	1.0000
46	<.0001	0.2917	0.7576	0.9999	<.0001	0.9893	1.0000	1.0000	1.0000	0.5991	0.0037	0.0001	1.0000	1.0000
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1064	<.0001	<.0001	<.0001	<.0001
48	1.0000	0.5991	0.1819	0.0077	1.0000	0.0311	<.0001	<.0001	0.0037	<.0001	1.0000	1.0000	0.0008	0.0017
49	0.8820	1.0000	0.9893	0.4352	0.9985	0.7576	0.0017	0.0003	0.2917	<.0001	1.0000	1.0000	0.1064	0.1819
50	0.1064	1.0000	1.0000	0.9984	0.2515	1.0000	0.1064	0.0311	0.9893	<.0001	1.0000	0.9893	0.8820	0.9569
51	1.0000	0.5991	0.1819	0.0077	1.0000	0.0311	<.0001	<.0001	0.0037	<.0001	1.0000	1.0000	0.0008	0.0017
52	0.0001	0.9999	1.0000	1.0000	0.0001	1.0000	0.9984	0.9569	1.0000	0.0077	0.4352	0.0589	1.0000	1.0000
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0311	<.0001	<.0001	<.0001	<.0001
54	<.0001	0.0311	0.1819	0.8820	<.0001	0.5991	1.0000	1.0000	0.9569	0.9893	0.0001	<.0001	0.9984	0.9893

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
1	<.0001	1.0000	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.9340	<.0001	<.0001	<.0001	0.0001	<.0001
2	0.0003	0.5991	0.1819	0.9893	0.7576	0.8820	0.9893	0.1064	0.9999	0.0589	0.0017	<.0001	0.9984	<.0001
3	0.5991	0.0003	<.0001	0.0077	0.0008	1.0000	1.0000	1.0000	0.0065	1.0000	0.8820	0.0001	1.0000	0.0001
4	0.9984	<.0001	<.0001	0.0001	<.0001	1.0000	1.0000	1.0000	<.0001	1.0000	1.0000	0.0077	0.9999	0.0077
5	0.1064	0.0077	0.0008	0.1064	0.0157	1.0000	1.0000	0.9893	0.1439	0.9569	0.2917	<.0001	1.0000	<.0001
6	1.0000	<.0001	<.0001	<.0001	<.0001	0.7576	0.4352	1.0000	<.0001	1.0000	1.0000	0.5991	0.2917	0.5991
7	<.0001	1.0000	0.9893	1.0000	1.0000	0.0589	0.1819	0.0008	1.0000	0.0003	<.0001	<.0001	0.2917	<.0001
8	<.0001	1.0000	1.0000	1.0000	1.0000	0.0077	0.0311	<.0001	1.0000	<.0001	<.0001	<.0001	0.0589	<.0001
9	0.9999	<.0001	<.0001	<.0001	<.0001	1.0000	0.9999	1.0000	<.0001	1.0000	1.0000	0.0157	0.9984	0.0157
10	1.0000	<.0001	<.0001	<.0001	<.0001	0.4352	0.1819	0.9984	<.0001	0.9999	1.0000	0.8820	0.1064	0.8820
11	1.0000	<.0001	<.0001	<.0001	<.0001	0.0157	0.0037	0.4352	<.0001	0.5991	0.9984	1.0000	0.0017	1.0000
12	<.0001	1.0000	1.0000	1.0000	1.0000	0.0017	0.0077	<.0001	1.0000	<.0001	<.0001	<.0001	0.0157	<.0001
13	<.0001	1.0000	1.0000	1.0000	1.0000	<.0001	0.0001	<.0001	0.8341	<.0001	<.0001	<.0001	0.0003	<.0001
14	<.0001	1.0000	1.0000	1.0000	1.0000	0.0003	0.0017	<.0001	0.9989	<.0001	<.0001	<.0001	0.0037	<.0001
15	<.0001	1.0000	1.0000	1.0000	1.0000	<.0001	0.0003	<.0001	0.9437	<.0001	<.0001	<.0001	0.0008	<.0001
16	0.0037	0.1819	0.0311	0.7576	0.2917	0.9984	1.0000	0.4352	0.9276	0.2917	0.0157	<.0001	1.0000	<.0001
17	0.0311	0.0311	0.0037	0.2917	0.0589	1.0000	1.0000	0.8820	0.4258	0.7576	0.1064	<.0001	1.0000	<.0001
18	0.4352	0.0008	<.0001	0.0157	0.0017	1.0000	1.0000	1.0000	0.0153	0.9999	0.7576	<.0001	1.0000	<.0001
19	<.0001	1.0000	1.0000	1.0000	1.0000	<.0001	0.0003	<.0001	0.9999	<.0001	<.0001	<.0001	0.0008	<.0001
20	0.1819	0.0037	0.0003	0.0589	0.0077	1.0000	1.0000	0.9984	0.0730	0.9893	0.4352	<.0001	1.0000	<.0001
21	1.0000	<.0001	<.0001	<.0001	<.0001	0.9999	0.9893	1.0000	<.0001	1.0000	1.0000	0.0589	0.9569	0.0589
22	1.0000	<.0001	<.0001	<.0001	<.0001	0.9893	0.8820	1.0000	<.0001	1.0000	1.0000	0.1819	0.7576	0.1819
23	0.5991	0.0003	<.0001	0.0077	0.0008	1.0000	1.0000	1.0000	0.0065	1.0000	0.8820	0.0001	1.0000	0.0001
24	1.0000	<.0001	<.0001	<.0001	<.0001	0.0157	0.0037	0.4352	<.0001	0.5991	0.9984	1.0000	0.0017	1.0000
25	<.0001	0.9893	0.7576	1.0000	0.9984	0.2917	0.5991	0.0077	1.0000	0.0037	<.0001	<.0001	0.7576	<.0001
26	<.0001	1.0000	0.9984	1.0000	1.0000	0.0311	0.1064	0.0003	1.0000	0.0001	<.0001	<.0001	0.1819	<.0001
27	0.8820	<.0001	<.0001	0.0017	0.0001	1.0000	1.0000	1.0000	0.0010	1.0000	0.9893	0.0008	1.0000	0.0008
28	0.7576	0.0001	<.0001	0.0037	0.0003	1.0000	1.0000	1.0000	0.0026	1.0000	0.9569	0.0003	1.0000	0.0003
29		<.0001	<.0001	<.0001	<.0001	0.5991	0.2917	0.9999	<.0001	1.0000	1.0000	0.7576	0.1819	0.7576
30	<.0001		1.0000	1.0000	1.0000	0.0003	0.0017	<.0001	0.9989	<.0001	<.0001	<.0001	0.0037	<.0001
31	<.0001	1.0000		1.0000	1.0000	<.0001	0.0001	<.0001	0.8341	<.0001	<.0001	<.0001	0.0003	<.0001
32	<.0001	1.0000	1.0000		1.0000	0.0077	0.0311	<.0001	1.0000	<.0001	<.0001	<.0001	0.0589	<.0001
33	<.0001	1.0000	1.0000	1.0000		0.0008	0.0037	<.0001	1.0000	<.0001	<.0001	<.0001	0.0077	<.0001
34	0.5991	0.0003	<.0001	0.0077	0.0008		1.0000	1.0000	0.0065	1.0000	0.8820	0.0001	1.0000	0.0001
35	0.2917	0.0017	0.0001	0.0311	0.0037	1.0000		0.9999	0.0345	0.9984	0.5991	<.0001	1.0000	<.0001
36	0.9999	<.0001	<.0001	<.0001	<.0001	1.0000	0.9999		<.0001	1.0000	1.0000	0.0157	0.9984	0.0157
37	<.0001	0.9989	0.8341	1.0000	1.0000	0.0065	0.0345	<.0001		<.0001	<.0001	<.0001	0.0730	<.0001
38	1.0000	<.0001	<.0001	<.0001	<.0001	1.0000	0.9984	1.0000	<.0001		1.0000	0.0311	0.9893	0.0311

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
39	1.0000	<.0001	<.0001	<.0001	<.0001	0.8820	0.5991	1.0000	<.0001	1.0000		0.4352	0.4352	0.4352
40	0.7576	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0157	<.0001	0.0311	0.4352		<.0001	1.0000
41	0.1819	0.0037	0.0003	0.0589	0.0077	1.0000	1.0000	0.9984	0.0730	0.9893	0.4352	<.0001		<.0001
42	0.7576	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0157	<.0001	0.0311	0.4352	1.0000	<.0001	
43	0.0017	0.2917	0.0589	0.8820	0.4352	0.9893	0.9999	0.2917	0.9835	0.1819	0.0077	<.0001	1.0000	<.0001
44	1.0000	<.0001	<.0001	<.0001	<.0001	0.8820	0.5991	1.0000	<.0001	1.0000	1.0000	0.4352	0.4352	0.4352
45	1.0000	<.0001	<.0001	<.0001	<.0001	0.9999	0.9893	1.0000	<.0001	1.0000	1.0000	0.0589	0.9569	0.0589
46	1.0000	<.0001	<.0001	<.0001	<.0001	1.0000	0.9984	1.0000	<.0001	1.0000	1.0000	0.0311	0.9893	0.0311
47	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.8820	<.0001	0.8820
48	<.0001	1.0000	1.0000	1.0000	1.0000	0.0037	0.0157	<.0001	1.0000	<.0001	<.0001	<.0001	0.0311	<.0001
49	<.0001	0.9893	0.7576	1.0000	0.9984	0.2917	0.5991	0.0077	1.0000	0.0037	<.0001	<.0001	0.7576	<.0001
50	0.0017	0.2917	0.0589	0.8820	0.4352	0.9893	0.9999	0.2917	0.9835	0.1819	0.0077	<.0001	1.0000	<.0001
51	<.0001	1.0000	1.0000	1.0000	1.0000	0.0037	0.0157	<.0001	1.0000	<.0001	<.0001	<.0001	0.0311	<.0001
52	0.4352	0.0008	<.0001	0.0157	0.0017	1.0000	1.0000	1.0000	0.0153	0.9999	0.7576	<.0001	1.0000	<.0001
53	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5991	<.0001	0.5991
54	1.0000	<.0001	<.0001	<.0001	<.0001	0.9569	0.7576	1.0000	<.0001	1.0000	1.0000	0.2917	0.5991	0.2917

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd												
i/j	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**
1	0.0632	<.0001	<.0001	<.0001	<.0001	1.0000	0.9086	0.0632	1.0000	<.0001	<.0001	<.0001
2	1.0000	0.0017	0.0311	0.0589	<.0001	0.9569	1.0000	1.0000	0.9569	0.9569	<.0001	0.0037
3	0.9893	0.8820	0.9999	1.0000	<.0001	0.0037	0.2917	0.9893	0.0037	1.0000	<.0001	0.9569
4	0.4352	1.0000	1.0000	1.0000	<.0001	<.0001	0.0157	0.4352	<.0001	1.0000	<.0001	1.0000
5	1.0000	0.2917	0.8820	0.9569	<.0001	0.0589	0.8820	1.0000	0.0589	1.0000	<.0001	0.4352
6	0.0037	1.0000	1.0000	1.0000	0.0003	<.0001	<.0001	0.0037	<.0001	0.5991	<.0001	1.0000
7	0.9984	<.0001	0.0001	0.0003	<.0001	1.0000	1.0000	0.9984	1.0000	0.1064	<.0001	<.0001
8	0.8820	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.8820	1.0000	0.0157	<.0001	<.0001
9	0.2917	1.0000	1.0000	1.0000	<.0001	<.0001	0.0077	0.2917	<.0001	1.0000	<.0001	1.0000
10	0.0008	1.0000	1.0000	0.9999	0.0017	<.0001	<.0001	0.0008	<.0001	0.2917	0.0003	1.0000
11	<.0001	0.9984	0.7576	0.5991	0.1064	<.0001	<.0001	<.0001	<.0001	0.0077	0.0311	0.9893
12	0.5991	<.0001	<.0001	<.0001	<.0001	1.0000	0.9999	0.5991	1.0000	0.0037	<.0001	<.0001
13	0.0589	<.0001	<.0001	<.0001	<.0001	1.0000	0.7576	0.0589	1.0000	<.0001	<.0001	<.0001
14	0.2917	<.0001	<.0001	<.0001	<.0001	1.0000	0.9893	0.2917	1.0000	0.0008	<.0001	<.0001
15	0.1064	<.0001	<.0001	<.0001	<.0001	1.0000	0.8820	0.1064	1.0000	0.0001	<.0001	<.0001
16	1.0000	0.0157	0.1819	0.2917	<.0001	0.5991	1.0000	1.0000	0.5991	0.9999	<.0001	0.0311
17	1.0000	0.1064	0.5991	0.7576	<.0001	0.1819	0.9893	1.0000	0.1819	1.0000	<.0001	0.1819

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	43	44	45	46	47	48	49	50	51	52	53	54
18	0.9984	0.7576	0.9984	0.9999	<.0001	0.0077	0.4352	0.9984	0.0077	1.0000	<.0001	0.8820
19	0.2515	<.0001	<.0001	<.0001	<.0001	1.0000	0.9985	0.2515	1.0000	0.0001	<.0001	<.0001
20	1.0000	0.4352	0.9569	0.9893	<.0001	0.0311	0.7576	1.0000	0.0311	1.0000	<.0001	0.5991
21	0.1064	1.0000	1.0000	1.0000	<.0001	<.0001	0.0017	0.1064	<.0001	0.9984	<.0001	1.0000
22	0.0311	1.0000	1.0000	1.0000	<.0001	<.0001	0.0003	0.0311	<.0001	0.9569	<.0001	1.0000
23	0.9893	0.8820	0.9999	1.0000	<.0001	0.0037	0.2917	0.9893	0.0037	1.0000	<.0001	0.9569
24	<.0001	0.9984	0.7576	0.5991	0.1064	<.0001	<.0001	<.0001	<.0001	0.0077	0.0311	0.9893
25	1.0000	<.0001	0.0017	0.0037	<.0001	1.0000	1.0000	1.0000	1.0000	0.4352	<.0001	0.0001
26	0.9893	<.0001	<.0001	0.0001	<.0001	1.0000	1.0000	0.9893	1.0000	0.0589	<.0001	<.0001
27	0.8820	0.9893	1.0000	1.0000	<.0001	0.0008	0.1064	0.8820	0.0008	1.0000	<.0001	0.9984
28	0.9569	0.9569	1.0000	1.0000	<.0001	0.0017	0.1819	0.9569	0.0017	1.0000	<.0001	0.9893
29	0.0017	1.0000	1.0000	1.0000	0.0008	<.0001	<.0001	0.0017	<.0001	0.4352	0.0001	1.0000
30	0.2917	<.0001	<.0001	<.0001	<.0001	1.0000	0.9893	0.2917	1.0000	0.0008	<.0001	<.0001
31	0.0589	<.0001	<.0001	<.0001	<.0001	1.0000	0.7576	0.0589	1.0000	<.0001	<.0001	<.0001
32	0.8820	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.8820	1.0000	0.0157	<.0001	<.0001
33	0.4352	<.0001	<.0001	<.0001	<.0001	1.0000	0.9984	0.4352	1.0000	0.0017	<.0001	<.0001
34	0.9893	0.8820	0.9999	1.0000	<.0001	0.0037	0.2917	0.9893	0.0037	1.0000	<.0001	0.9569
35	0.9999	0.5991	0.9893	0.9984	<.0001	0.0157	0.5991	0.9999	0.0157	1.0000	<.0001	0.7576
36	0.2917	1.0000	1.0000	1.0000	<.0001	<.0001	0.0077	0.2917	<.0001	1.0000	<.0001	1.0000
37	0.9835	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.9835	1.0000	0.0153	<.0001	<.0001
38	0.1819	1.0000	1.0000	1.0000	<.0001	<.0001	0.0037	0.1819	<.0001	0.9999	<.0001	1.0000
39	0.0077	1.0000	1.0000	1.0000	0.0001	<.0001	<.0001	0.0077	<.0001	0.7576	<.0001	1.0000
40	<.0001	0.4352	0.0589	0.0311	0.8820	<.0001	<.0001	<.0001	<.0001	<.0001	0.5991	0.2917
41	1.0000	0.4352	0.9569	0.9893	<.0001	0.0311	0.7576	1.0000	0.0311	1.0000	<.0001	0.5991
42	<.0001	0.4352	0.0589	0.0311	0.8820	<.0001	<.0001	<.0001	<.0001	<.0001	0.5991	0.2917
43		0.0077	0.1064	0.1819	<.0001	0.7576	1.0000	1.0000	0.7576	0.9984	<.0001	0.0157
44	0.0077		1.0000	1.0000	0.0001	<.0001	<.0001	0.0077	<.0001	0.7576	<.0001	1.0000
45	0.1064	1.0000		1.0000	<.0001	<.0001	0.0017	0.1064	<.0001	0.9984	<.0001	1.0000
46	0.1819	1.0000	1.0000		<.0001	<.0001	0.0037	0.1819	<.0001	0.9999	<.0001	1.0000
47	<.0001	0.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
48	0.7576	<.0001	<.0001	<.0001	<.0001		1.0000	0.7576	1.0000	0.0077	<.0001	<.0001
49	1.0000	<.0001	0.0017	0.0037	<.0001	1.0000		1.0000	1.0000	0.4352	<.0001	0.0001
50	1.0000	0.0077	0.1064	0.1819	<.0001	0.7576	1.0000		0.7576	0.9984	<.0001	0.0157
51	0.7576	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.7576		0.0077	<.0001	<.0001
52	0.9984	0.7576	0.9984	0.9999	<.0001	0.0077	0.4352	0.9984	0.0077		<.0001	0.8820
53	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
54	0.0157	1.0000	1.0000	1.0000	<.0001	<.0001	0.0001	0.0157	<.0001	0.8820	<.0001	

LSMEAN for milks with 10 g/L starch. Treatment 1: 63; Treatment 2: 63 held for 30 min; Treatment 3: 72 . Starch numbers reference Table 1. Starch 0 means control- no starch added

treatment	starch	curd LSMEAN	LSMEAN Number
1	0	13.0000000	1
1	1	17.7500000	2
1	2	24.0000000	3
1	3	16.2500000	4
1	4	25.2500000	5
1	5	34.5000000	6
1	6	20.2500000	7
1	7	15.7500000	8
1	8	26.7500000	9
1	9	32.0000000	10
1	10	37.2500000	11
1	11	13.7500000	12
1	12	11.2500000	13
1	13	11.5000000	14
1	14	10.7500000	15
1	15	20.7500000	16
1	16	40.2500000	17
1	17	26.0000000	18
2	0	12.5000000	19
2	1	21.5000000	20
2	2	28.0000000	21
2	3	32.2500000	22
2	4	25.0000000	23
2	5	35.0000000	24
2	6	20.2500000	25
2	7	18.5000000	26
2	8	28.5000000	27
2	9	28.0000000	28
2	10	45.2500000	29
2	11	16.0000000	30
2	12	11.7500000	31
2	13	12.5000000	32
2	14	11.5000000	33
2	15	22.7500000	34

treatment	starch	curd LSMEAN	LSMEAN Number
2	**16**	42.7500000	35
2	**17**	28.2500000	36
3	**0**	14.2500000	37
3	**1**	25.0000000	38
3	**2**	30.0000000	39
3	**3**	35.2500000	40
3	**4**	25.5000000	41
3	**5**	35.7500000	42
3	**6**	24.2500000	43
3	**7**	29.2500000	44
3	**8**	30.2500000	45
3	**9**	31.0000000	46
3	**10**	47.0000000	47
3	**11**	16.5000000	48
3	**12**	13.7500000	49
3	**13**	19.5000000	50
3	**14**	20.7500000	51
3	**15**	29.0000000	52
3	**16**	49.7500000	53
3	**17**	26.5000000	54

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.3867	<.0001	0.9784	<.0001	<.0001	0.0026	0.9989	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
2	0.3867		0.2367	1.0000	0.0361	<.0001	1.0000	1.0000	0.0021	<.0001	<.0001	0.9768	0.1709	0.2367
3	<.0001	0.2367		0.0233	1.0000	<.0001	0.9916	0.0092	1.0000	0.0147	<.0001	0.0001	<.0001	<.0001
4	0.9784	1.0000	0.0233		0.0021	<.0001	0.9768	1.0000	<.0001	<.0001	<.0001	1.0000	0.7346	0.8262
5	<.0001	0.0361	1.0000	0.0021		0.0012	0.7346	0.0007	1.0000	0.1201	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	<.0001	<.0001	0.0012		<.0001	<.0001	0.0233	1.0000	1.0000	<.0001	<.0001	<.0001
7	0.0026	1.0000	0.9916	0.9768	0.7346	<.0001		0.8979	0.1709	<.0001	<.0001	0.1709	0.0021	0.0034
8	0.9989	1.0000	0.0092	1.0000	0.0007	<.0001	0.8979		<.0001	<.0001	<.0001	1.0000	0.8979	0.9474
9	<.0001	0.0021	1.0000	<.0001	1.0000	0.0233	0.1709	<.0001		0.6296	<.0001	<.0001	<.0001	<.0001
10	<.0001	<.0001	0.0147	<.0001	0.1201	1.0000	<.0001	<.0001	0.6296		0.6296	<.0001	<.0001	<.0001
11	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.6296		<.0001	<.0001	<.0001
12	1.0000	0.9768	0.0001	1.0000	<.0001	<.0001	0.1709	1.0000	<.0001	<.0001	<.0001		1.0000	1.0000
13	1.0000	0.1709	<.0001	0.7346	<.0001	<.0001	0.0021	0.8979	<.0001	<.0001	<.0001	1.0000		1.0000
14	1.0000	0.2367	<.0001	0.8262	<.0001	<.0001	0.0034	0.9474	<.0001	<.0001	<.0001	1.0000	1.0000	
15	1.0000	0.0822	<.0001	0.5199	<.0001	<.0001	0.0007	0.7346	<.0001	<.0001	<.0001	0.9999	1.0000	1.0000

Least Squares Means for effect treatment*starch Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: curd														
i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
16	0.0007	0.9999	0.9995	0.8979	0.8979	<.0001	1.0000	0.7346	0.3181	<.0001	<.0001	0.0822	0.0007	0.0012
17	<.0001	<.0001	<.0001	<.0001	<.0001	0.4138	<.0001	<.0001	<.0001	0.0092	0.9999	<.0001	<.0001	<.0001
18	<.0001	0.0092	1.0000	0.0004	1.0000	0.0056	0.4138	0.0001	1.0000	0.3181	<.0001	<.0001	<.0001	<.0001
19	1.0000	0.1878	<.0001	0.8738	<.0001	<.0001	0.0007	0.9784	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
20	<.0001	0.9916	1.0000	0.6296	0.9916	<.0001	1.0000	0.4138	0.6296	<.0001	<.0001	0.0233	0.0001	0.0002
21	<.0001	0.0001	0.9768	<.0001	1.0000	0.1709	0.0233	<.0001	1.0000	0.9768	0.0012	<.0001	<.0001	<.0001
22	<.0001	<.0001	0.0092	<.0001	0.0822	1.0000	<.0001	<.0001	0.5199	1.0000	0.7346	<.0001	<.0001	<.0001
23	<.0001	0.0551	1.0000	0.0034	1.0000	0.0007	0.8262	0.0012	1.0000	0.0822	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	0.0004	1.0000	<.0001	<.0001	0.0092	0.9999	1.0000	<.0001	<.0001	<.0001
25	0.0026	1.0000	0.9916	0.9768	0.7346	<.0001	1.0000	0.8979	0.1709	<.0001	<.0001	0.1709	0.0021	0.0034
26	0.1225	1.0000	0.5199	1.0000	0.1201	<.0001	1.0000	1.0000	0.0092	<.0001	<.0001	0.8262	0.0551	0.0822
27	<.0001	<.0001	0.8979	<.0001	0.9995	0.3181	0.0092	<.0001	1.0000	0.9976	0.0034	<.0001	<.0001	<.0001
28	<.0001	0.0001	0.9768	<.0001	1.0000	0.1709	0.0233	<.0001	1.0000	0.9768	0.0012	<.0001	<.0001	<.0001
29	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0147	<.0001	<.0001	<.0001
30	0.9940	1.0000	0.0147	1.0000	0.0012	<.0001	0.9474	1.0000	<.0001	<.0001	<.0001	1.0000	0.8262	0.8979
31	1.0000	0.3181	<.0001	0.8979	<.0001	<.0001	0.0056	0.9768	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
32	1.0000	0.6296	<.0001	0.9916	<.0001	<.0001	0.0233	0.9995	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
33	1.0000	0.2367	<.0001	0.8262	<.0001	<.0001	0.0034	0.9474	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
34	<.0001	0.7346	1.0000	0.1709	1.0000	<.0001	1.0000	0.0822	0.9768	0.0012	<.0001	0.0021	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	0.0092	<.0001	<.0001	<.0001	<.0001	0.5199	<.0001	<.0001	<.0001
36	<.0001	<.0001	0.9474	<.0001	0.9999	0.2367	0.0147	<.0001	1.0000	0.9916	0.0021	<.0001	<.0001	<.0001
37	1.0000	0.9415	<.0001	1.0000	<.0001	<.0001	0.0468	1.0000	<.0001	<.0001	<.0001	1.0000	0.9940	0.9989
38	<.0001	0.0551	1.0000	0.0034	1.0000	0.0007	0.8262	0.0012	1.0000	0.0822	<.0001	<.0001	<.0001	<.0001
39	<.0001	<.0001	0.3181	<.0001	0.8262	0.8979	0.0004	<.0001	0.9995	1.0000	0.0551	<.0001	<.0001	<.0001
40	<.0001	<.0001	<.0001	<.0001	0.0002	1.0000	<.0001	<.0001	0.0056	0.9995	1.0000	<.0001	<.0001	<.0001
41	<.0001	0.0233	1.0000	0.0012	1.0000	0.0021	0.6296	0.0004	1.0000	0.1709	<.0001	<.0001	<.0001	<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	0.0021	0.9916	1.0000	<.0001	<.0001	<.0001
43	<.0001	0.1709	1.0000	0.0147	1.0000	0.0001	0.9768	0.0056	1.0000	0.0233	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	0.6296	<.0001	0.9768	0.6296	0.0021	<.0001	1.0000	1.0000	0.0147	<.0001	<.0001	<.0001
45	<.0001	<.0001	0.2367	<.0001	0.7346	0.9474	0.0002	<.0001	0.9976	1.0000	0.0822	<.0001	<.0001	<.0001
46	<.0001	<.0001	0.0822	<.0001	0.4138	0.9976	<.0001	<.0001	0.9474	1.0000	0.2367	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	<.0001	<.0001	<.0001
48	0.9415	1.0000	0.0361	1.0000	0.0034	<.0001	0.9916	1.0000	0.0001	<.0001	<.0001	1.0000	0.6296	0.7346
49	1.0000	0.9768	0.0001	1.0000	<.0001	<.0001	0.1709	1.0000	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000
50	0.0158	1.0000	0.8979	0.9995	0.4138	<.0001	1.0000	0.9916	0.0551	<.0001	<.0001	0.4138	0.0092	0.0147
51	0.0007	0.9999	0.9995	0.8979	0.8979	<.0001	1.0000	0.7346	0.3181	<.0001	<.0001	0.0822	0.0007	0.0012
52	<.0001	<.0001	0.7346	<.0001	0.9916	0.5199	0.0034	<.0001	1.0000	0.9999	0.0092	<.0001	<.0001	<.0001

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
54	<.0001	0.0034	1.0000	0.0001	1.0000	0.0147	0.2367	<.0001	1.0000	0.5199	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1.0000	0.0007	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.0026	0.1225	<.0001	<.0001
2	0.0822	0.9999	<.0001	0.0092	0.1878	0.9916	0.0001	<.0001	0.0551	<.0001	1.0000	1.0000	<.0001	0.0001
3	<.0001	0.9995	<.0001	1.0000	<.0001	1.0000	0.9768	0.0092	1.0000	<.0001	0.9916	0.5199	0.8979	0.9768
4	0.5199	0.8979	<.0001	0.0004	0.8738	0.6296	<.0001	<.0001	0.0034	<.0001	0.9768	1.0000	<.0001	<.0001
5	<.0001	0.8979	<.0001	1.0000	<.0001	0.9916	1.0000	0.0822	1.0000	0.0004	0.7346	0.1201	0.9995	1.0000
6	<.0001	<.0001	0.4138	0.0056	<.0001	<.0001	0.1709	1.0000	0.0007	1.0000	<.0001	<.0001	0.3181	0.1709
7	0.0007	1.0000	<.0001	0.4138	0.0007	1.0000	0.0233	<.0001	0.8262	<.0001	1.0000	1.0000	0.0092	0.0233
8	0.7346	0.7346	<.0001	0.0001	0.9784	0.4138	<.0001	<.0001	0.0012	<.0001	0.8979	1.0000	<.0001	<.0001
9	<.0001	0.3181	<.0001	1.0000	<.0001	0.6296	1.0000	0.5199	1.0000	0.0092	0.1709	0.0092	1.0000	1.0000
10	<.0001	<.0001	0.0092	0.3181	<.0001	<.0001	0.9768	1.0000	0.0822	0.9999	<.0001	<.0001	0.9976	0.9768
11	<.0001	<.0001	0.9999	<.0001	<.0001	<.0001	0.0012	0.7346	<.0001	1.0000	<.0001	<.0001	0.0034	0.0012
12	0.9999	0.0822	<.0001	<.0001	1.0000	0.0233	<.0001	<.0001	<.0001	<.0001	0.1709	0.8262	<.0001	<.0001
13	1.0000	0.0007	<.0001	<.0001	1.0000	0.0001	<.0001	<.0001	<.0001	<.0001	0.0021	0.0551	<.0001	<.0001
14	1.0000	0.0012	<.0001	<.0001	1.0000	0.0002	<.0001	<.0001	<.0001	<.0001	0.0034	0.0822	<.0001	<.0001
15		0.0002	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	0.0233	<.0001	<.0001
16	0.0002		<.0001	0.6296	0.0002	1.0000	0.0551	<.0001	0.9474	<.0001	1.0000	1.0000	0.0233	0.0551
17	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	0.0147	<.0001	0.6296	<.0001	<.0001	<.0001	<.0001
18	<.0001	0.6296	<.0001		<.0001	0.8979	1.0000	0.2367	1.0000	0.0021	0.4138	0.0361	1.0000	1.0000
19	1.0000	0.0002	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	0.0468	<.0001	<.0001
20	<.0001	1.0000	<.0001	0.8979	<.0001		0.1709	<.0001	0.9976	<.0001	1.0000	0.9999	0.0822	0.1709
21	<.0001	0.0551	<.0001	1.0000	<.0001	0.1709		0.9474	0.9999	0.0822	0.0233	0.0007	1.0000	1.0000
22	<.0001	<.0001	0.0147	0.2367	<.0001	<.0001	0.9474		0.0551	1.0000	<.0001	<.0001	0.9916	0.9474
23	<.0001	0.9474	<.0001	1.0000	<.0001	0.9976	0.9999	0.0551		0.0002	0.8262	0.1709	0.9976	0.9999
24	<.0001	<.0001	0.6296	0.0021	<.0001	<.0001	0.0822	1.0000	0.0002		<.0001	<.0001	0.1709	0.0822
25	0.0007	1.0000	<.0001	0.4138	0.0007	1.0000	0.0233	<.0001	0.8262	<.0001		1.0000	0.0092	0.0233
26	0.0233	1.0000	<.0001	0.0361	0.0468	0.9999	0.0007	<.0001	0.1709	<.0001	1.0000		0.0002	0.0007
27	<.0001	0.0233	<.0001	1.0000	<.0001	0.0822	1.0000	0.9916	0.9976	0.1709	0.0092	0.0002		1.0000
28	<.0001	0.0551	<.0001	1.0000	<.0001	0.1709	1.0000	0.9474	0.9999	0.0822	0.0233	0.0007	1.0000	
29	<.0001	<.0001	0.7346	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001
30	0.6296	0.8262	<.0001	0.0002	0.9415	0.5199	<.0001	<.0001	0.0021	<.0001	0.9474	1.0000	<.0001	<.0001
31	1.0000	0.0021	<.0001	<.0001	1.0000	0.0004	<.0001	<.0001	<.0001	<.0001	0.0056	0.1201	<.0001	<.0001

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
32	1.0000	0.0092	<.0001	<.0001	1.0000	0.0021	<.0001	<.0001	<.0001	<.0001	0.0233	0.3181	<.0001	<.0001
33	1.0000	0.0012	<.0001	<.0001	1.0000	0.0002	<.0001	<.0001	<.0001	<.0001	0.0034	0.0822	<.0001	<.0001
34	<.0001	1.0000	<.0001	0.9995	<.0001	1.0000	0.6296	0.0007	1.0000	<.0001	1.0000	0.9474	0.4138	0.6296
35	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0233	<.0001	<.0001	<.0001	<.0001
36	<.0001	0.0361	<.0001	1.0000	<.0001	0.1201	1.0000	0.9768	0.9995	0.1201	0.0147	0.0004	1.0000	1.0000
37	0.9415	0.0158	<.0001	<.0001	0.9999	0.0026	<.0001	<.0001	<.0001	<.0001	0.0468	0.6491	<.0001	<.0001
38	<.0001	0.9474	<.0001	1.0000	<.0001	0.9976	0.9999	0.0551	1.0000	0.0002	0.8262	0.1709	0.9976	0.9999
39	<.0001	0.0012	0.0001	0.9768	<.0001	0.0056	1.0000	1.0000	0.7346	0.7346	0.0004	<.0001	1.0000	1.0000
40	<.0001	<.0001	0.7346	0.0012	<.0001	<.0001	0.0551	0.9999	0.0001	1.0000	<.0001	<.0001	0.1201	0.0551
41	<.0001	0.8262	<.0001	1.0000	<.0001	0.9768	1.0000	0.1201	1.0000	0.0007	0.6296	0.0822	0.9999	1.0000
42	<.0001	<.0001	0.8979	0.0004	<.0001	<.0001	0.0233	0.9976	<.0001	1.0000	<.0001	<.0001	0.0551	0.0233
43	<.0001	0.9976	<.0001	1.0000	<.0001	1.0000	0.9916	0.0147	1.0000	<.0001	0.9768	0.4138	0.9474	0.9916
44	<.0001	0.0056	<.0001	0.9995	<.0001	0.0233	1.0000	0.9999	0.9474	0.4138	0.0021	<.0001	1.0000	1.0000
45	<.0001	0.0007	0.0002	0.9474	<.0001	0.0034	1.0000	1.0000	0.6296	0.8262	0.0002	<.0001	1.0000	1.0000
46	<.0001	0.0001	0.0012	0.7346	<.0001	0.0007	0.9999	1.0000	0.3181	0.9768	<.0001	<.0001	1.0000	0.9999
47	<.0001	<.0001	0.1201	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	0.4138	0.9474	<.0001	0.0007	0.7737	0.7346	<.0001	<.0001	0.0056	<.0001	0.9916	1.0000	<.0001	<.0001
49	0.9999	0.0822	<.0001	<.0001	1.0000	0.0233	<.0001	<.0001	<.0001	<.0001	0.1709	0.8262	<.0001	<.0001
50	0.0034	1.0000	<.0001	0.1709	0.0049	1.0000	0.0056	<.0001	0.5199	<.0001	1.0000	1.0000	0.0021	0.0056
51	0.0002	1.0000	<.0001	0.6296	0.0002	1.0000	0.0551	<.0001	0.9474	<.0001	1.0000	1.0000	0.0233	0.0551
52	<.0001	0.0092	<.0001	0.9999	<.0001	0.0361	1.0000	0.9995	0.9768	0.3181	0.0034	<.0001	1.0000	1.0000
53	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
54	<.0001	0.4138	<.0001	1.0000	<.0001	0.7346	1.0000	0.4138	1.0000	0.0056	0.2367	0.0147	1.0000	1.0000

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	<.0001	0.9940	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001	1.0000	0.3181	0.6296	0.2367	0.7346	<.0001	<.0001	0.9415	0.0551	<.0001	<.0001	0.0233	<.0001
3	<.0001	0.0147	<.0001	<.0001	<.0001	1.0000	<.0001	0.9474	<.0001	1.0000	0.3181	<.0001	1.0000	<.0001
4	<.0001	1.0000	0.8979	0.9916	0.8262	0.1709	<.0001	<.0001	1.0000	0.0034	<.0001	<.0001	0.0012	<.0001
5	<.0001	0.0012	<.0001	<.0001	<.0001	1.0000	<.0001	0.9999	<.0001	1.0000	0.8262	0.0002	1.0000	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0092	0.2367	<.0001	0.0007	0.8979	1.0000	0.0021	1.0000
7	<.0001	0.9474	0.0056	0.0233	0.0034	1.0000	<.0001	0.0147	0.0468	0.8262	0.0004	<.0001	0.6296	<.0001
8	<.0001	1.0000	0.9768	0.9995	0.9474	0.0822	<.0001	<.0001	1.0000	0.0012	<.0001	<.0001	0.0004	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001	0.9768	<.0001	1.0000	<.0001	1.0000	0.9995	0.0056	1.0000	0.0021
10	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012	<.0001	0.9916	<.0001	0.0822	1.0000	0.9995	0.1709	0.9916

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
11	0.0147	<.0001	<.0001	<.0001	<.0001	<.0001	0.5199	0.0021	<.0001	<.0001	0.0551	1.0000	<.0001	1.0000
12	<.0001	1.0000	1.0000	1.0000	1.0000	0.0021	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	0.8262	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.9940	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	0.8979	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.9989	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	0.6296	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.9415	<.0001	<.0001	<.0001	<.0001	<.0001
16	<.0001	0.8262	0.0021	0.0092	0.0012	1.0000	<.0001	0.0361	0.0158	0.9474	0.0012	<.0001	0.8262	<.0001
17	0.7346	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0001	0.7346	<.0001	0.8979
18	<.0001	0.0002	<.0001	<.0001	<.0001	0.9995	<.0001	1.0000	<.0001	1.0000	0.9768	0.0012	1.0000	0.0004
19	<.0001	0.9415	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.9999	<.0001	<.0001	<.0001	<.0001	<.0001
20	<.0001	0.5199	0.0004	0.0021	0.0002	1.0000	<.0001	0.1201	0.0026	0.9976	0.0056	<.0001	0.9768	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	0.6296	<.0001	1.0000	<.0001	0.9999	1.0000	0.0551	1.0000	0.0233
22	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	<.0001	0.9768	<.0001	0.0551	1.0000	0.9999	0.1201	0.9976
23	<.0001	0.0021	<.0001	<.0001	<.0001	1.0000	<.0001	0.9995	<.0001	1.0000	0.7346	0.0001	1.0000	<.0001
24	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0233	0.1201	<.0001	0.0002	0.7346	1.0000	0.0007	1.0000
25	<.0001	0.9474	0.0056	0.0233	0.0034	1.0000	<.0001	0.0147	0.0468	0.8262	0.0004	<.0001	0.6296	<.0001
26	<.0001	1.0000	0.1201	0.3181	0.0822	0.9474	<.0001	0.0004	0.6491	0.1709	<.0001	<.0001	0.0822	<.0001
27	<.0001	<.0001	<.0001	<.0001	<.0001	0.4138	<.0001	1.0000	<.0001	0.9976	1.0000	0.1201	0.9999	0.0551
28	<.0001	<.0001	<.0001	<.0001	<.0001	0.6296	<.0001	1.0000	<.0001	0.9999	1.0000	0.0551	1.0000	0.0233
29		<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	0.0007
30	<.0001		0.9474	0.9976	0.8979	0.1201	<.0001	<.0001	1.0000	0.0021	<.0001	<.0001	0.0007	<.0001
31	<.0001	0.9474		1.0000	1.0000	<.0001	<.0001	<.0001	0.9999	<.0001	<.0001	<.0001	<.0001	<.0001
32	<.0001	0.9976	1.0000		1.0000	0.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001
33	<.0001	0.8979	1.0000	1.0000		<.0001	<.0001	<.0001	0.9989	<.0001	<.0001	<.0001	<.0001	<.0001
34	<.0001	0.1201	<.0001	0.0001	<.0001		<.0001	0.5199	<.0001	1.0000	0.0551	<.0001	1.0000	<.0001
35	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	0.0361	<.0001	0.0822
36	<.0001	<.0001	<.0001	<.0001	<.0001	0.5199	<.0001		<.0001	0.9995	1.0000	0.0822	1.0000	0.0361
37	<.0001	1.0000	0.9999	1.0000	0.9989	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	0.0021	<.0001	<.0001	<.0001	1.0000	<.0001	0.9995	<.0001		0.7346	0.0001	1.0000	<.0001
39	<.0001	<.0001	<.0001	<.0001	<.0001	0.0551	<.0001	1.0000	<.0001	0.7346		0.6296	0.8979	0.4138
40	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	0.0361	0.0822	<.0001	0.0001	0.6296		0.0004	1.0000
41	<.0001	0.0007	<.0001	<.0001	<.0001	1.0000	<.0001	1.0000	<.0001	1.0000	0.8979	0.0004		0.0001
42	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	0.0822	0.0361	<.0001	<.0001	0.4138	1.0000	0.0001	
43	<.0001	0.0092	<.0001	<.0001	<.0001	1.0000	<.0001	0.9768	<.0001	1.0000	0.4138	<.0001	1.0000	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	0.1709	<.0001	1.0000	<.0001	0.9474	1.0000	0.3181	0.9916	0.1709
45	<.0001	<.0001	<.0001	<.0001	<.0001	0.0361	<.0001	1.0000	<.0001	0.6296	1.0000	0.7346	0.8262	0.5199
46	<.0001	<.0001	<.0001	<.0001	<.0001	0.0092	<.0001	1.0000	<.0001	0.3181	1.0000	0.9474	0.5199	0.8262
47	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.9474	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	1.0000	0.8262	0.9768	0.7346	0.2367	<.0001	<.0001	1.0000	0.0056	<.0001	<.0001	0.0021	<.0001

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
49	<.0001	1.0000	1.0000	1.0000	1.0000	0.0021	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001
50	<.0001	0.9976	0.0233	0.0822	0.0147	0.9995	<.0001	0.0034	0.1878	0.5199	<.0001	<.0001	0.3181	<.0001
51	<.0001	0.8262	0.0021	0.0092	0.0012	1.0000	<.0001	0.0361	0.0158	0.9474	0.0012	<.0001	0.8262	<.0001
52	<.0001	<.0001	<.0001	<.0001	<.0001	0.2367	<.0001	1.0000	<.0001	0.9768	1.0000	0.2367	0.9976	0.1201
53	0.8979	<.0001	<.0001	<.0001	<.0001	<.0001	0.0822	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
54	<.0001	<.0001	<.0001	<.0001	<.0001	0.9916	<.0001	1.0000	<.0001	1.0000	0.9976	0.0034	1.0000	0.0012

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	43	44	45	46	47	48	49	50	51	52	53	54
1	<.0001	<.0001	<.0001	<.0001	<.0001	0.9415	1.0000	0.0158	0.0007	<.0001	<.0001	<.0001
2	0.1709	<.0001	<.0001	<.0001	<.0001	1.0000	0.9768	1.0000	0.9999	<.0001	<.0001	0.0034
3	1.0000	0.6296	0.2367	0.0822	<.0001	0.0361	0.0001	0.8979	0.9995	0.7346	<.0001	1.0000
4	0.0147	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.9995	0.8979	<.0001	<.0001	0.0001
5	1.0000	0.9768	0.7346	0.4138	<.0001	0.0034	<.0001	0.4138	0.8979	0.9916	<.0001	1.0000
6	0.0001	0.6296	0.9474	0.9976	<.0001	<.0001	<.0001	<.0001	<.0001	0.5199	<.0001	0.0147
7	0.9768	0.0021	0.0002	<.0001	<.0001	0.9916	0.1709	1.0000	1.0000	0.0034	<.0001	0.2367
8	0.0056	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.9916	0.7346	<.0001	<.0001	<.0001
9	1.0000	1.0000	0.9976	0.9474	<.0001	0.0001	<.0001	0.0551	0.3181	1.0000	<.0001	1.0000
10	0.0233	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.9999	<.0001	0.5199
11	<.0001	0.0147	0.0822	0.2367	0.0004	<.0001	<.0001	<.0001	<.0001	0.0092	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.4138	0.0822	<.0001	<.0001	<.0001
13	<.0001	<.0001	<.0001	<.0001	<.0001	0.6296	1.0000	0.0092	0.0007	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	0.7346	1.0000	0.0147	0.0012	<.0001	<.0001	<.0001
15	<.0001	<.0001	<.0001	<.0001	<.0001	0.4138	0.9999	0.0034	0.0002	<.0001	<.0001	<.0001
16	0.9976	0.0056	0.0007	0.0001	<.0001	0.9474	0.0822	1.0000	1.0000	0.0092	<.0001	0.4138
17	<.0001	<.0001	0.0002	0.0012	0.1201	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	<.0001
18	1.0000	0.9995	0.9474	0.7346	<.0001	0.0007	<.0001	0.1709	0.6296	0.9999	<.0001	1.0000
19	<.0001	<.0001	<.0001	<.0001	<.0001	0.7737	1.0000	0.0049	0.0002	<.0001	<.0001	<.0001
20	1.0000	0.0233	0.0034	0.0007	<.0001	0.7346	0.0233	1.0000	1.0000	0.0361	<.0001	0.7346
21	0.9916	1.0000	1.0000	0.9999	<.0001	<.0001	<.0001	0.0056	0.0551	1.0000	<.0001	1.0000
22	0.0147	0.9999	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.9995	<.0001	0.4138
23	1.0000	0.9474	0.6296	0.3181	<.0001	0.0056	<.0001	0.5199	0.9474	0.9768	<.0001	1.0000
24	<.0001	0.4138	0.8262	0.9768	<.0001	<.0001	<.0001	<.0001	<.0001	0.3181	<.0001	0.0056
25	0.9768	0.0021	0.0002	<.0001	<.0001	0.9916	0.1709	1.0000	1.0000	0.0034	<.0001	0.2367
26	0.4138	<.0001	<.0001	<.0001	<.0001	1.0000	0.8262	1.0000	1.0000	<.0001	<.0001	0.0147
27	0.9474	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.0021	0.0233	1.0000	<.0001	1.0000

Least Squares Means for effect treatment*starch
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: curd

i/j	43	44	45	46	47	48	49	50	51	52	53	54
28	0.9916	1.0000	1.0000	0.9999	<.0001	<.0001	<.0001	0.0056	0.0551	1.0000	<.0001	1.0000
29	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	0.8979	<.0001
30	0.0092	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.9976	0.8262	<.0001	<.0001	<.0001
31	<.0001	<.0001	<.0001	<.0001	<.0001	0.8262	1.0000	0.0233	0.0021	<.0001	<.0001	<.0001
32	<.0001	<.0001	<.0001	<.0001	<.0001	0.9768	1.0000	0.0822	0.0092	<.0001	<.0001	<.0001
33	<.0001	<.0001	<.0001	<.0001	<.0001	0.7346	1.0000	0.0147	0.0012	<.0001	<.0001	<.0001
34	1.0000	0.1709	0.0361	0.0092	<.0001	0.2367	0.0021	0.9995	1.0000	0.2367	<.0001	0.9916
35	<.0001	<.0001	<.0001	<.0001	0.9474	<.0001	<.0001	<.0001	<.0001	<.0001	0.0822	<.0001
36	0.9768	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.0034	0.0361	1.0000	<.0001	1.0000
37	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000	0.1878	0.0158	<.0001	<.0001	<.0001
38	1.0000	0.9474	0.6296	0.3181	<.0001	0.0056	<.0001	0.5199	0.9474	0.9768	<.0001	1.0000
39	0.4138	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	0.0012	1.0000	<.0001	0.9976
40	<.0001	0.3181	0.7346	0.9474	<.0001	<.0001	<.0001	<.0001	<.0001	0.2367	<.0001	0.0034
41	1.0000	0.9916	0.8262	0.5199	<.0001	0.0021	<.0001	0.3181	0.8262	0.9976	<.0001	1.0000
42	<.0001	0.1709	0.5199	0.8262	<.0001	<.0001	<.0001	<.0001	<.0001	0.1201	<.0001	0.0012
43		0.7346	0.3181	0.1201	<.0001	0.0233	<.0001	0.8262	0.9976	0.8262	<.0001	1.0000
44	0.7346		1.0000	1.0000	<.0001	<.0001	<.0001	0.0004	0.0056	1.0000	<.0001	1.0000
45	0.3181	1.0000		1.0000	<.0001	<.0001	<.0001	<.0001	0.0007	1.0000	<.0001	0.9916
46	0.1201	1.0000	1.0000		<.0001	<.0001	<.0001	<.0001	0.0001	1.0000	<.0001	0.8979
47	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
48	0.0233	<.0001	<.0001	<.0001	<.0001		1.0000	0.9999	0.9474	<.0001	<.0001	0.0002
49	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000		0.4138	0.0822	<.0001	<.0001	<.0001
50	0.8262	0.0004	<.0001	<.0001	<.0001	0.9999	0.4138		1.0000	0.0007	<.0001	0.0822
51	0.9976	0.0056	0.0007	0.0001	<.0001	0.9474	0.0822	1.0000		0.0092	<.0001	0.4138
52	0.8262	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	0.0007	0.0092		<.0001	1.0000
53	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
54	1.0000	1.0000	0.9916	0.8979	<.0001	0.0002	<.0001	0.0822	0.4138	1.0000	<.0001	